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intralytix
SAFETY BY NATURE®

August 14, 2020

Susan J. Carlson, Ph.D.
Division of Food Ingredients
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Ref: Intralytix GRAS Notification for CampyShield™

Dear Dr. Carlson,

In accordance with the Federal Register [81 Fed. Reg. 159 (17 August 2016)] issuance of GRAS notifications (21CFR§170), Intralytix is pleased to submit a notice that we have concluded, through scientific procedures, the bacteriophage cocktail CampyShield™ is generally recognized as safe and is not subject to pre-market approval requirements for the use in foods, generally, as a processing aid to control *Campylobacter jejuni*.

We request that a copy of the notification is shared with the United States Department of Agriculture's Food Safety and Inspection Service, regarding the use of CampyShield™ as a safe and suitable antimicrobial used in the production of meat and poultry products as a processing aid.

CampyShield™ is substantially equivalent to several other bacteriophage products previously granted GRAS status by the FDA and listed in FSIS Directive 7120.1 as processing aids.

Please let me know if you have any questions or comments.

Sincerely,

Alexander Sulakvelidze, Ph.D.

Vice President & Chief Scientific Officer
Intralytix, Inc.
PHONE: 410-625-2533
EMAIL: asulakvelidze@intralytix.com

1 SIGNED STATEMENTS AND CERTIFICATION

1.1 STATEMENT OF INTENT

In accordance with the 21 CFR 170 Subpart E, regulations for GRAS notifications, Intralytix is pleased to submit a notice that we have concluded, through scientific procedures, the bacteriophage preparation, CampyShield™, is generally recognized as safe and is not subject to the premarket approval requirements for the use in foods, generally, as a processing aid to control *Campylobacter* under the intended use conditions described within this notification.

1.2 NAME & ADDRESS OF NOTIFIER

Intralytix, Inc.
8681 Robert Fulton Dr.
Columbia, MD 21046
Tel: 877-489-7424
Fax: 410-625-2506

1.3 COMMON OR USUAL NAME

Intralytix produces a lytic bacteriophage preparation with potent lytic activity against the Gram-negative bacterium *Campylobacter* under the trade name CampyShield™.

1.4 CONDITIONS OF USE

CampyShield™ is intended for use as an antimicrobial to control *Campylobacter* spp. on food when applied to food surfaces up to $1x10^8$ PFU / gram of food, including the following food categories:

- Raw and ground poultry
- Raw red meat products

1.5 BASIS FOR THE GRAS CONCLUSION

Pursuant to the GRAS rule, Intralytix has concluded that CampyShield™ is GRAS through scientific procedures, in accordance with 21 CFR 170.30 (a) and (b).

1.6 CAMPYSHIELD IS NOT SUBJECT TO PREMARKET APPROVAL

Because Intralytix has concluded that CampyShield™ is GRAS, it is not subject to the premarket approval requirements for the use in foods, generally, as a processing aid to control *Campylobacter* under the intended use conditions described within this notification.

1.7 AVAILABILITY OF INFORMATION

The data and information that are the basis for Intralytix's conclusion that CampyShield™ is GRAS are available for review and copying by FDA during customary business hours, at the location below, or will be sent to FDA upon request, made to:

Intralytix
Joelle Woolston
8681 Robert Fulton Dr.
Columbia, MD 21046
jwoolston@intralytix.com

1.8 FREEDOM OF INFORMATION ACT

It is our view that the information contained in this notification is not exempt from disclosure under the Freedom of Information Act.

1.9 CERTIFICATION

To the best of our knowledge, this GRAS notification is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of CampyShield™.

1.10 SIGNATURE

Alexander Sulakvelidze, Ph.D.
Chief Scientific Officer
asulakvelidze@intralytix.com

Date: August 14, 2020

1.11 FSIS AUTHORIZATION

We also request that a copy of the notification be shared with the United States Department of Agriculture's Food Safety and Inspection Service, regarding the use of CampyShield™ as a safe and suitable antimicrobial used in the production of meat and poultry products as a processing aid. CampyShield™ is substantially equivalent to several other bacteriophage products also listed in FSIS Directive 7120.1 as processing aids.

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2 IDENTITY AND SPECIFICATIONS OF CAMPYSHIELD™

2.1 IDENTITY

CampyShield is a mixture of three to eight bacteriophages (phages or monophages) targeting *Campylobacter*. The specific purpose of mixing three to eight monophages is to enable quick response to real-life situations when uncommon / new *Campylobacter* strains may be emerging and contaminating food products. The ability to utilize various blends of up to eight lytic phages allows our phage biocontrol technology to ensure optimal efficacy which, in turn, is critical for ensuring the safety of foods. Namely, it helps in (i) warranting the broadest possible lytic activity of the cocktail for effective control of various *Campylobacter* strains, and (ii) reducing the risk of development of bacterial resistance against CampyShield.

The product may be safely used as an antimicrobial in accordance with the following conditions[1]:

1) The phages are produced on host *Campylobacter* strains grown in animal-product free media.

2) The titer of each monophage in the cocktail is ≥ 9.0 $\log_{10}$ PFU/mL and the titer of the cocktail is ≥ 10.0 $\log_{10}$ PFU/mL.

3) The phages do not contain a functional portion of any of the toxin-encoding sequences described in 40 CFR 725.421(d).

4) The phages do not contain sequences derived from genes encoding bacterial 16S ribosomal RNA.

5) The cocktail consists of a mixture of approximately equal proportions of three to eight different individually purified bacteriophages lytic against *Campylobacter*.

6) The *Campylobacter* host strains used for production do not encode any functional toxin genes.

7) The cocktail achieves positive lytic results by a spot titer assay against one or more *Campylobacter* strains available in reference collections (e.g. ATCC).

8) The cocktail contains ≤ 25,000 EU/mL of endotoxin at a concentration of bacteriophages 9.0 $\log_{10}$ PFU/mL.

9) The cocktail is determined to be bacteriologically sterile.

10) The phage cocktail is used in accordance with the conditions of use outlines in Section 1.4.

CampyShield is a concentrate that is normally diluted with water at the application site to form the CampyShield working solution, typically with a lytic titer of ca. 9.0 $\log_{10}$ PFU/mL. It is applied

[1] The inclusion criteria for CampyShield are the same ten criteria for inclusion set forth in the EcoShield PX (GRN 834) that granted flexibility for EcoShield PX.

at a rate that ensures the final concentration of phage on the food articles is at or below $1x10^8$ PFU/g of food.

2.2 METHOD OF MANUFACTURE

The component monophages of CampyShield are prepared using Intralytix's well-established phage production protocols. These procedures have been reviewed by the FDA for manufacturing of Intralytix's bacteriophage food safety products, most recently in GRN 834. For CampyShield, the individual monophages are each produced using a microaerobic process, in separate production runs.

For each monophage, the host *Campylobacter jejuni* strain is grown to a target concentration (CFU/mL), at which point the culture is infected with the monophage at a previously determined MOI (multiplicity of infection; the ratio of phage to bacteria) and the combination is incubated in microaerophilic conditions. The suspension is clarified by removal of bacteria by filtration. Following the initial filtration, the monophages are concentrated, washed with Phosphate Buffered Saline (PBS), and then sterilized using filtration. After each of the component monophages has passed quality control specifications, proper volumes of each monophage, and sterile PBS as necessary, are combined, and final filtration is carried out using a sterilizing grade filter. The CampyShield article of commerce is prepared so that:

Each monophage is approximately equally represented

AND

The lytic titer is ≥10.0 $\log_{10}$ PFU/mL

The CampyShield article of commerce is typically diluted with clean water at the application site, to form the "working solution" or "working concentration" of CampyShield with a lytic titer of 9.0 $\log_{10}$ PFU/mL.

The filters used in the production of CampyShield are all constructed of component materials that are non-toxic and are compliant with the criteria of USP <88> for Biological Reactivity for USP Class VI plastics. The component materials are listed by the FDA as appropriate for use in articles intended for repeated food contact. Additionally, the filters comply with 21 CFR § 210.3(b)(6) as non-fiber releasing. The final fill containers are made of food-grade materials and are compliant with 21 CFR § 177.1315 (bottle) and 21 CFR § 177.1520 (closure).

Figure 1 provides an overall schematic of the process.

2.3 SPECIFICATIONS

Due to the two-step manufacturing process, there are two levels of quality control. First, each individual monophage lot is analyzed to ensure it meets the release specifications listed in Table 1 before it can be used to prepare a lot of CampyShield.

Table 1 Product specifications for individual monophage lots

Parameter	Specification
Potency (PFU/mL)	≥10.0 $\log_{10}$ PFU/mL
Microbial purity	No growth
Identity	Matches reference

Only after all component monophages have met the release specifications can a lot of CampyShield be produced. Each lot of CampyShield is analyzed to ensure it meets the following release specifications listed in Table 2.

Table 2 Product specifications for CampyShield

Parameter	Specification
Potency (PFU/mL)	≥10.0 $\log_{10}$ PFU/mL
Microbial purity	No growth
Endotoxin Content (EU/mL)	≤25,000 EU/mL (at ca. 9.0 $\log_{10}$ PFU/mL)
Identity Test	All component phages are present

2.4 CHARACTERISTIC PROPERTIES

CampyShield is a clear to opalescent odorless liquid. The phage component of CampyShield (typical working concentration of ca. $1x10^9$ PFU/mL) is roughly estimated to be 0.000035% by weight and the remainder is PBS. Typical chemical analysis of CampyShield (at the typical working concentration of ca. $1x10^9$ PFU/mL) is shown below. The values shown are derived (averages) from the chemical analysis of three separate CampyShield lots.

Table 3 Typical chemical analysis of CampyShield

Property/analysis/composition	Reporting Detection Limit	CampyShield Lot# 0420G2103A38	CampyShield Lot# 0420G2103B27	CampyShield Lot# 0420G2103C95	CampyShield average
pH	n/a	7.1	7.1	n/d	7.1
Arsenic (mg/L)	0.01	ND	ND	ND	ND
Barium (mg/L)	0.01	ND	ND	ND	ND
Cadmium (mg/L)	0.0025	ND	ND	ND	ND
Calcium (mg/L)	0.5	ND	ND	ND	ND
Chromium (mg/L)	0.02	ND	ND	ND	ND
Cobalt (mg/L)	0.01	ND	ND	ND	ND
Copper (mg/L))	0.01	ND	ND	ND	ND
Iron (mg/L)	0.05	ND	ND	ND	ND
Lead (mg/L)	0.01	ND	ND	ND	ND

Property/analysis/composition	Reporting Detection Limit	CampyShield Lot# 0420G2103A38	CampyShield Lot# 0420G2103B27	CampyShield Lot# 0420G2103C95	CampyShield average
Magnesium (mg/L)	0.5	ND	ND	ND	ND
Manganese (mg/L)	0.05	ND	ND	ND	ND
Molybdenum (mg/L)	0.05	ND	ND	ND	ND
Nickel (mg/L)	0.01	ND	ND	ND	ND
Potassium (mg/L)	0.5	86	57	64	69
Selenium (mg/L)	0.05	ND	ND	ND	ND
Sodium (mg/L)	10	2280	1080	1344	1568
Tin (mg/L)	0.02	ND	ND	ND	ND
Zinc (mg/L)	0.05	ND	ND	ND	ND

n/a = not applicable; n/d = not determined; ND = none detected

2.5 PHAGE CLASSIFICATION

The current component phages in CampyShield were fully characterized by a variety of methods, including electron microscopy (EM), whole-genome sequence analysis, and lytic activity against *Campylobacter* strains and non-*Campylobacter* strains.

The three component bacteriophages currently included in CampyShield are listed below:

Name: J350
Order: Caudovirales
Family: Myoviridae
Properties: Double-stranded DNA, Lytic

Name: J375
Order: Caudovirales
Family: Myoviridae
Properties: Double-stranded DNA, Lytic

Name: J386
Order: Caudovirales
Family: Myoviridae
Properties: Double-stranded DNA, Lytic

The monophages have not been genetically manipulated (i.e., not GMO).

2.6 POTENTIAL HUMAN TOXICANTS

The *Campylobacter* host strains, as with all Gram-negative bacteria, produce bacterial endotoxin or lipopolysaccharide (LPS). Intralytix tests every lot of CampyShield for LPS to ensure it meets the release criteria. Endotoxins are further discussed below, in Sections 3.1.2.3, 6.1.3, and 6.2.1.2.

Similar to other Enterobacteria, certain *Campylobacter* strains are known to carry an enterotoxin, cytolethal distending toxin (CDT). Even though great care is taken to remove media products, processing enzymes, and host material – including nucleic acids – from phage lysates, bacterial strains that may be used for phage propagation are routinely screened for enterotoxins. The *C. jejuni* host strains used for propagation do not contain the functionally active genes for any of the known enterotoxins. *Campylobacter* toxins are further discussed in Section 6.2.1.2.

2.7 STABILITY

The proposed shelf life of CampyShield article of commerce is one year when stored at 2-8°C in a dark, UV-protected area.

3 DIETARY EXPOSURE

3.1 APPLICATION RATES AND DIETARY INTAKE

3.1.1 APPLICATION RATES

The current CampyShield article of commerce is a concentrate that is typically diluted with water at the application site to form the CampyShield working solution. It is applied at a rate that ensures the final concentration of phage on the food articles is at or below $1x10^8$ PFU/g of food. Future preparations may be sold in more concentrated form, but the accompanying instructions for dilution and application rate will be appropriately adjusted to ensure the final concentration of phage on the food articles is always at or below $1x10^8$ PFU/g of food.

3.1.2 DIETARY INTAKES

CampyShield is envisioned to be used upon foods, including those in the following food categories:

1) Raw and ground poultry

2) Raw red meat products

The calculations described in the subsequent sections were performed to estimate the dietary intake of CampyShield when used at the maximum application of $1x10^8$ PFU/g for each of the above food categories.

To determine the daily intake of each of the food categories for the US population as a whole, the Food Availability (Per Capita) Data System, provided by the United States Department of Agriculture's Economic Research Services was used (1). The per capita usage is a measure of food disappearance that is calculated by dividing the total supply available, after accounting for spoilage and waste, by the US population.

All calculations below are based on a maximum (worst-case scenario) consumption of CampyShield. This worst-case scenario assumes 100% market saturation (i.e., that the entire food supply is treated with CampyShield) and that the maximum application rate of $1x10^8$ PFU/g is used. Even with the added margin of safety added by these overestimations, the amounts of CampyShield, and its constituents, that would be consumed via the two food categories are very small, as shown in the following calculations.

3.1.2.1 DIETARY INTAKES FOR CAMPYSHIELD

The following calculation to determine the maximum (worst-case scenario) consumption of CampyShield by the average American uses the maximum application of CampyShield ($1x10^8$ PFU/g). The concentration recommended for the working solution of CampyShield is

1x10^9 PFU/mL. Using that concentration, the volume of CampyShield that would be applied per gram of treated food can be calculated as follows:

$$\frac{1 \times 10^8\ PFU}{g\ food} \times \frac{1\ mL\ CampyShield}{1 \times 10^9\ PFU} = \frac{0.1\ mL\ CampyShield}{g\ food}$$

Using 0.1mL CampyShield applied per gram of food, the volume of CampyShield that would be consumed per day via each food category can be calculated and is presented in Table 4. Assuming the worst-case scenario, where 100% of the foods in the two food groups were treated at the maximum application (1x10^8 PFU/g), the combined total amount of CampyShield consumed per day would be about 17 mL or just over 1 tablespoon.

Table 4 Volume of CampyShield consumed per day when applied at 1x10^8 PFU/g food

	Consumed per American per day* (g)	CampyShield consumed per person per day (mL)
Poultry	75.1	7.5
Red meat	91.9	9.2
Total of all categories	167.0	16.7

* The per capita consumption data was obtained from Food Availability (Per Capita) Data System (1). The loss adjusted availability of total poultry and total red meat in grams/day for most recent available data (2017) were used in the calculations.

The majority of the 1 Tbsp of CampyShield would constitute water; the phages, sodium, and potassium contained within that approximate 1 Tbsp would be negligible, as evidenced by the dietary calculations presented below.

3.1.2.2 DIETARY INTAKES FOR CAMPYSHIELD PHAGES

The following calculation determines the approximate weight of phages consumed per day, again assuming the maximum CampyShield application (1x10^8 PFU/g):

Total phages (PFU) consumed per day:

$$\frac{1 \times 10^8\ PFU}{g\ food} \times \frac{167\ g\ food}{day} = \frac{1.67 \times 10^{10}\ PFU}{day}$$

Weight of total phages consumed/day (in micrograms):

$$\frac{1.67 \times 10^{10}\ PFU}{day} \times \frac{3.49 \times 10^{-16}\ g}{phage} \times \frac{1 \times 10^6\ \mu g}{g} = \frac{5.8\ \mu g}{day}$$

Where 3.49 x10^{-16} g = mass of one phage

Assuming the average diet is 3 kg/day, the dietary concentration of phages is:

$$\frac{5.8\ \mu g}{day} \times \frac{1\ day}{3\ kg} = 1.9\ ppb$$

The weight of phages consumed per day via CampyShield would be 5.8 μg, or 1.9 ppb in a 3 kg diet. This is insignificant.

3.1.2.3 DIETARY INTAKE OF ENDOTOXIN

Normal saliva contains approximately 1 mg endotoxin per mL (2). For endotoxin, 1 EU/mL is approximately equal to 1 ng/mL. This means that the 1 mg/mL of endotoxin in saliva is equivalent to approximately $1x10^6$ EU/mL. Specification for CampyShield lots for endotoxin is ≤ 25,000 EU/mL at $1x10^9$ PFU/mL.

The approximate daily volume of CampyShield consumed is approximately 16.7 mL (see Section 3.1.2.1). Again, using the worst-case scenario (maximum allowable endotoxin level by specification), the maximum amount of endotoxin consumed via CampyShield is thus:

$$\frac{16.7\ mL\ CampyShield}{day} \times \frac{2.5\ \times 10^4\ EU}{mL\ CampyShield} = \frac{4.2\ \times 10^5\ EU}{day}$$

Humans produce approximately 500 to 750 mL of saliva per day. Using the lower, more conservative number, healthy humans consume from saliva:

$$\frac{500\ mL\ saliva}{day} \times \frac{1\ \times 10^6\ EU}{mL\ saliva} = \frac{5 \times 10^8\ EU}{day}$$

The maximal amount contributed by CampyShield would thus constitute 0.084 % of the daily load of endotoxin from saliva. The level of endotoxin found in CampyShield is therefore considered safe.

3.1.2.4 SODIUM AND POTASSIUM CONTENT

From Section 2.4, the highest value obtained for sodium content in a CampyShield lot was 2280 mg/L. From this value and using the worst-case scenario value from Table 4 (all foods from each food category are treated with CampyShield), the amount of sodium contributed to the daily diet via CampyShield can be calculated as follows:

$$\frac{2280\ mg\ sodium}{1000\ mL\ CampyShield} \times \frac{16.7\ mL\ CampyShield}{day} = \frac{38.1\ mg\ sodium}{day}$$

The recommended daily allowance of sodium is 2,300 mg (21 CFR § 101.9(c)(9)). The amount of sodium per day contributed by CampyShield thus represents 1.7 % of the RDA and is negligible. The amount of sodium per day contributed by CampyShield, 38.1 mg, would be spread across several servings and meals. The amount of sodium consumed per serving would likely be below the level that would change nutritional content labeling by the end-user.

From Section 2.4, highest value obtained for potassium content in a CampyShield lot was 86 mg/L. From this value, the amount of potassium contributed to the daily diet via CampyShield on the targeted food categories can be calculated as follows:

$$\frac{86\ mg\ potassium}{1000\ mL\ CampyShield} \times \frac{16.7\ mL\ CampyShield}{day} = \frac{1.4\ mg\ potassium}{day}$$

The recommended daily allowance of potassium is 4,700 mg (21 CFR § 101.9(c)(9)). The amount of potassium per day contributed by CampyShield, 1.4 mg, is well below the level that would change nutritional content labeling by the end-user and represents 0.03% of the RDA.

4 SELF-LIMITING LEVELS OF USE

The proposed use for CampyShield is as an antimicrobial processing aid for foods that are at high risk to be contaminated with *Campylobacter*.

The self-limiting levels of use are:

- Due to the cost of the product, the end-user would use the minimum dose required to achieve a significant reduction or elimination of *Campylobacter*.
- Once the *Campylobacter* contamination is depleted, the phage will slowly decrease in number due to a lack of host.
- Phages are susceptible to many environmental factors, including sunlight, heat, and UV light. Exposure to these will cause the number of phages to decrease.

5 EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

This section is not applicable to this notification.

6 NARRATIVE

In the following sections, the data and information providing the basis for our conclusion that CampyShield is GRAS, through scientific procedures, under the conditions of its intended use is presented. The information provided below, and elsewhere in this document, that is generally available has been properly cited. The list of references is presented in Part 7.

6.1 COMPONENTS OF CAMPYSHIELD

CampyShield is a mixture of component bacteriophages together with added PBS; due to the method of production, there may also be small amounts of residual production by-products. The primary active ingredient is not a single chemical substance but a mixture of naturally occurring bacteriophages. In the appropriate sections below, we consider separately the safety of the:

- Phages (active component)
- Added salts
- Manufacturing by-products

6.1.1 MONOPHAGES

The safety and ubiquity of bacteriophages have been well established. The pertinent safety data on bacteriophages is reviewed below. The published literature on phages and other information developed by Intralytix show that:

- Bacteriophages are the most ubiquitous organisms on earth. Their population densities are estimated to be in the range of 10^7 to 10^9 plaque forming units per gm or per mL depending upon the matrix under consideration (3, 4) and the total number of phages in the biosphere has been estimated to be in the range of 10^{30}-10^{32} (5). This abundance of phages in the environment, and the continuous exposure of animals to them, explains the extremely good tolerance of mammalian organisms to phages.

- The biology of phages has been exhaustively studied. The studies have clearly shown that the lytic bacteriophages are obligate intracellular parasites of bacteria and are not infectious in humans or other mammals (6, 7).

- Bacteriophages are commensals of the human gastrointestinal tract (GIT), and play an important role in regulating the diversity and community structure of the GIT microbiome (8). A high concentration of DNA bacteriophages of the taxonomic Order Caudovirales is reported from the human GIT (9). Recently, a novel bacteriophage "crAssphage" was identified in human fecal samples (10) and is shown to be abundant and ubiquitous in the mammalian GIT, including the human GIT (11).

- Phages specific to *E. coli, Bacteroides fragilis*, and various *Salmonella* serotypes have been isolated from human fecal specimens in concentrations as high as 10^5 PFU/100 g of feces (8, 12-14). The recent data based on metagenomic analyses (using partial shotgun sequencing) of an uncultured viral community from human feces suggested that bacteriophages are the second most abundant category after bacteria in the uncultured fecal library (15, 16) with an estimated 10^{12}-10^{15} phages typically present in the human gastrointestinal tract (17, 18).

- The recent evidence suggest that GIT phages may offer human health benefits. Specifically, the abundance and diversity of GIT phages is associated with improved human health (19, 20).

- Phages have been used therapeutically in humans for almost 100 years, without any serious side effects (6, 21). During the long history of therapeutic use in Eastern Europe and the former Soviet Union (and, before the antibiotic era, in the United States, France, Australia, and other countries), phages have been administered to humans in various forms:

    - orally, in tablet or liquid formulations,
    - rectally,
    - locally (skin, eye, ear, nasal mucosa, etc.); in tampons, rinses, and creams,
    - as aerosols or intrapleural injections, and
    - intravenously

- Critical analysis of in-vitro and in-vivo studies did not reveal any harmful effects or serious complications because of bacteriophage use in humans including children (22). On the contrary, the available data indicate phages may exert anti-inflammatory and downregulate hyperactive immune system effects and may help protect overall health (22, 23). Recent reviews summarize the results of some of the human therapy studies involving bacteriophages (22, 24-30).

- Phages have also been administered to humans for non-therapeutic purposes without any adverse effects. For example, phage preparations have been used extensively to monitor humoral immune functions in humans including patients with Down's syndrome, the Wiskott-Aldrich syndrome, and immunodeficient patients in the United States in the 1970s-1990s (31, 32). In some studies (including several studies performed by the FDA), the purified phages were injected intravenously into HIV-infected patients or other immunodeficient individuals without any apparent side effects (33-35).

- Phages have also been administered to humans via various sera and FDA-approved vaccines commercially available in the United States (35-38).

- A recent study suggests that humans absorb up to 30 billion phages every day (39).
- No serious adverse immunologic or allergic sequelae have ever been reported because of human or animal exposure to phages (6, 25).
- Bacteriophages are commonly consumed via drinking water (40, 41).
- Bacteriophages are natural components of all fresh, unprocessed foods and are commonly consumed via various foods. For example, bacteriophages have been readily isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (42-56). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophages. For instance, bacteriophages were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed by Kennedy, Oblinger (48). The levels ranged from 3.3 to $4.4x10^{10}$ PFU/100 g of fresh chicken, up to $3.5x10^{10}$ PFU/100 g of fresh pork, and up to $2.7x10^{10}$ PFU/100 g of roast turkey breast samples. Additionally, *E. coli*- and *Shigella*-specific bacteriophages were recently isolated from 100% of beef and 68% of mixed salad purchased in a variety of markets (57). *Campylobacter* specific phages have been isolated from retail chicken livers (58).
- Because of their (i) highly specific nature and (ii) everyday exposure to humans (including daily consumption of bacteriophages with various foods and drinking water) and animals, bacteriophages do not deleteriously affect the GI microflora. For example:
    - In a trial to determine safety of bacteriophage application, oral administration of narrow host-range *E. coli*-specific phage T4 to 15 healthy adult volunteers did not elicit any adverse effects and was well-tolerated (59). Additionally, no substantial phage replication was detected. It did not cause a decrease in total fecal *E. coli* counts upon oral administration. Since the T4 phage was highly specific, no substantial phage T4 replication on the commensal *E. coli* population was identified, and no adverse events related to phage application were observed in any of the volunteers (59).
    - A pharmacokinetic and toxicological study using mice and guinea pigs did not show any signs of acute toxicity or histological changes, even when the dose administered was 3500-fold higher than the human dose projected in the course of the study (60).
    - High doses of *Listeria* phage preparations (i.e., ListShield and P100) were administered to laboratory animals (mice and rats) without any adverse effects (61, 62).

- A long-term toxicity study with a *Shigella* phage preparation (under the tradename ShigActive) in mice, showed no significant effect on any health or toxicity markers in the mice. Additionally, the phage preparation did not significantly affect the microbiota of the treated mice (63).

- Bacteriophages are commonly consumed by animals (including agriculturally-important species) via various foods. For example, in a study from Texas A&M University, male-specific and somatic coliphages were detected in all animal feeds, feed ingredients, and poultry diets examined, even after the samples were stored at -20°C for 14 months (64).

6.1.1.1 LYTIC PHAGES ARE GRAS

All lytic phages are, by nature, GRAS. There are two major types of phages: "virulent" (also called "lytic") and "temperate" (often mistakenly called "lysogenic"). Lytic phages lyse host bacteria without integrating into the host genome. In contrast, temperate phages may integrate into the host genome and a small subset of these may theoretically transduce undesirable bacterial genes, such as those encoding toxins or antibiotic resistance. Both lytic and temperate phages are ubiquitous in the environment, including the human and animal gut, the human oral cavity, foods sold at retail, sewage, and many other places that we encounter daily. Humans shed large numbers of both lytic and temperate phages into the environment every day – estimated to be on the order of $4x10^9$ phages daily per person (21). Temperate phages are found in almost all bacterial genera, including *Staphylococcus*, *Vibrio*, *Pseudomonas*, *Salmonella*, *Shigella*, *Bacillus*, *Corynebacterium*, *Listeria,* and *Streptococcus* (65-68). Indeed, some strains can release as many as five different types of temperate phages. Although the possibility of added gene transfer events is highly unlikely to bring danger to any individual consuming temperate phages, the use of such phages on an industrial scale could increase the overall risk of potentially harmful genes being acquired by new bacterial strains. Therefore, Intralytix identifies and uses only lytic phages in its phage preparations (including CampyShield).

6.1.1.2 CAMPYSHIELD MONOPHAGES ARE GRAS

The component phages in CampyShield were each characterized by various approaches, including electron microscopy, genotypic fingerprinting, and full genome sequence analysis. The component phages in CampyShield are members of the *Myoviridae* double-stranded DNA phage families, as defined by the International Committee on the Taxonomy of Viruses (ICTV) and by Ackermann et al. (69).

Intralytix will fully sequence any and all component monophages included in CampyShield. This approach is used to exclude bacteriophages carrying sequences encoding undesirable genes, and phages displaying prior evidence of transduction (e.g., bacterial 16s RNA genes).

Intralytix excludes all bacteriophages carrying sequences encoding any undesirable genes. Undesirable genes include genes encoding bacterial toxins (including genes listed in 40 CFR § 725.421), other known toxin genes, and genes associated with drug resistance. Undesirable genes are identified by comparing a complete bacteriophage sequence to all sequences

contained in GenBank and other databases available through the National Center for Biotechnology Information website of the National Library of Medicine using the BLASTn program (http://www.ncbi.nlm.nih.gov/BLAST/).

The cut-off *e*-value level for the latter analysis is $1x10^{-4}$, which detects virtually all undesirable genes in the phages' genomes. In practice, significant matches are considered to be those with *e*-values of $\leq 10^{-5}$ (70). Therefore, our proposed cut-off value provides a very strong (10-fold higher than the proposed 10^{-5} cut-off) assurance that undesirable genes are not missed during the analysis.

Intralytix will sequence the complete genome of each phage incorporated into CampyShield. Table 5 summarizes the current three phage genomes' properties. Analysis of the sequences yielded the following results:

- No toxin genes have been identified among the open reading frames of the annotated genomes of any of the three monophages.
- No 16S ribosomal RNA genes have been identified among annotated genomes of any of the three monophages.
- No antibiotic resistance genes have been identified among annotated genomes of any of the three monophages.

Summary: The approach of obtaining the full nucleotide sequence for each commercialized phage and complete bioinformatics analysis of all open reading frames will ensure that no detrimental genes will be present in any of the phages which will be used. This provides the fullest assurance of the phage safety as can presently be obtained by any method.

Table 5 Genome size and composition of phages contained in CampyShield

Phage	GC%	Size (bp)	Number of Open Reading Frames (ORFs)	Undesirable genes
J350	26.5	149,041	144	None
J375	26.2	123,818	159	None
J386	27.4	164,023	182	None

6.1.1.3 CAMPYSHIELD IS SPECIFIC TO CAMPYLOBACTER

Lytic activity of CampyShield monophages is targeted against *Campylobacter* strains. CampyShield has been screened for its lytic activity against 61 *Campylobacter jejuni* isolates in the Intralytix collection. As shown in Table 6, CampyShield is effective against the collection.

Table 6 *C. jejuni* in Intralytix's collection and the percent susceptible to CampyShield

Species	# isolates in Intralytix collection	Percent kill by CampyShield™	
		$2x10^4$ PFU/mL	$1x10^9$ PFU/mL
C. jejuni	61	43%	67%

CampyShield is also highly specific. CampyShield™ and the component monophages do not lyse any of the non-targeted bacterial strains (Table 7). These strains include both Gram-positive, five each of *Staphylococcus* and *Listeria*, and Gram-negative, five each of *Pseudomonas*, *Salmonella*, and *Escherichia*, bacterial species.

Table 7 Lytic activity of CampyShield against strains of common bacteria

Non-*C. jejuni* isolates		Species	Susceptibility to CampyShield™ ($1x10^9$ PFU/mL)
Intralytix ID	Original ID		
SA-36	ATCC 25923	*Staphylococcus aureus*	-
SA-37	ATCC 29213	*Staphylococcus aureus*	-
SA-211	ATCC 700699	*Staphylococcus aureus*	-
SA-298	ATCC 49775	*Staphylococcus aureus*	-
SA-299	ATCC 14458	*Staphylococcus aureus*	-
Lm 314	ATCC 19117	*Listeria monocytogenes*	-
Lm 315	ATCC 19118	*Listeria monocytogenes*	-
L.innocua 316	ATCC 51724	*Listeria innocua*	-
Lm 317	ATCC 19116	*Listeria monocytogenes*	-
L.innocua 318	ATCC 33090	*Listeria innocua*	-
Pa76	ATCC 10145	*Pseudomonas aeruginosa*	-
Pa161	ATCC 15692	*Pseudomonas aeruginosa*	-
Pa162	ATCC 51674	*Pseudomonas aeruginosa*	-
Pa163	ATCC 43390	*Pseudomonas aeruginosa*	-
Pa164	ATCC 39324	*Pseudomonas aeruginosa*	-
S.E566	ATCC 13076	*Salmonella Enteritidis*	-
S.T567	ATCC 13311	*Salmonella Typhimurium*	-
S.H568	ATCC 51956	*Salmonella Hadar*	-
S.T795	ATCC 19585	*Salmonella Typhimurium*	-
S.He899	ATCC 8326	*Salmonella Heidelberg*	-
Ec147	ATCC 43895	*Escherichia coli O157:H7*	-
Ec148	ATCC 35401	*Escherichia coli O78:H11*	-
Ec150	ATCC 700728	*Escherichia coli O157:H7*	-
Ec154	ATCC 11303	*Escherichia coli*	-
Ec155	ATCC 12435	*Escherichia coli*	-

6.1.2 ADDED SALTS: PHOSPHATE BUFFERED SALINE

The phosphate buffered saline (PBS) used in the manufacturing contains the following:

Sodium chloride: Sodium chloride "table salt" is the prototype in 21 CFR § 182.1 (a) of an ingredient that is so obviously GRAS that the FDA has not listed it as GRAS.

Potassium chloride: Potassium chloride is GRAS as described in 21 CFR § 582.5622.

Sodium phosphate dibasic: Sodium phosphate (mono-, di-, and tribasic) is affirmed GRAS as described in 21 CFR §182.1778, when used in accordance with good manufacturing practice.

Potassium phosphate monobasic: Potassium phosphate monobasic is approved as a food ingredient for use in the preparation of meat products including red meat and poultry, 9 CFR § 421.21(c) to decrease the amount of cooked out juices.

6.1.3 BY-PRODUCTS

Even though great care is taken to remove media products, processing enzymes, and host material – including nucleic acids – from phage lysates, bacterial strains that may be used for phage propagation are routinely screened for enterotoxins. Certain *C. jejuni* isolates are reported to produce cytolethal distending toxin (CDT) similar to other Enterobacteriaceae such as *E. coli* (71). The CDT is a heat labile toxin and is readily inactivated by cooking (71). The current host strains have been determined to lack a functional gene for the enterotoxin. The CDT is further discussed in Section 6.2.1.2.

The *C. jejuni* host strains are Gram-negative bacteria. As with all Gram-negative bacteria, they produce bacterial endotoxin or LPS. Intralytix tests every lot of CampyShield to ensure its LPS levels fall below the established release criteria. Endotoxins are further discussed in Sections 6.2.1.2 and 6.2.2.2.

6.2 MANUFACTURING OF CAMPYSHIELD™

CampyShield is manufactured using Intralytix's standard procedures. These procedures have been reviewed by the FDA for the manufacturing of several of Intralytix's bacteriophage food safety products, ListShield (21 CFR §172.785), EcoShield (FCN No. 1018), EcoShield PX (GRN 834), SalmoFresh (GRN 435), and ShigaShield (GRN 672) and are currently used to manufacture commercial lots of these products.

CampyShield is prepared by cultivation of individual host *Campylobacter* strain/phage combinations followed by filtration, concentration, wash, and final sterile filtration. After each monophage passes quality control, the monophages are combined with Phosphate Buffered Saline to form the CampyShield concentrate. Final filtration is then carried out with a sterilizing grade filter.

6.2.1 STARTING MATERIALS

There are four starting materials for manufacture of CampyShield component monophages:

Animal-product free media

Host strain

Monophages

Antifoam (as needed)

The safety of each is considered separately below.

6.2.1.1 ANIMAL-PRODUCT FREE MEDIA

A vegan media is used for bacterial growth and phage propagation. The main components are described here and have an existing regulatory status as GRAS or affirmed as food or for food application(s).

Peptone (Vegetable): Peptones are GRAS affirmed in 21 CFR § 184.1553 for use as processing aids, among other uses, at levels not to exceed good manufacturing practice. Peptones are protein hydrolysates consisting of free amino acids and short peptides in an aqueous salt solution.

Vegetable infusion powder and Vegetable special infusion powder: Both of these products are dehydrated infusion obtained from vegetable proteins. Vegetable proteins and its products are Food as described in 21 CFR § 170.3(n)(33).

Sodium phosphate dibasic: Sodium phosphate (mono-, di-, and tribasic) is affirmed GRAS as described in 21 CFR §182.1778, when used in accordance with good manufacturing practice.

Sodium chloride: Sodium chloride "table salt" is the prototype in 21 CFR § 182.1 (a) of an ingredient that is so obviously GRAS that FDA has not listed it as GRAS.

Dextrose: Dextrose, commonly known as corn sugar, is GRAS affirmed in 21 CFR § 184.1857 to be used in food with no limitation other than good manufacturing practice.

6.2.1.2 HOST STRAINS

The component monophages are produced on *Campylobacter jejuni* strains from Intralytix's collection of *Campylobacter* strains. These *C. jejuni* host strains have been characterized at Intralytix. Their biochemical properties were examined using the bioMérieux API CAMPY testing kit and their genomic composition has been examined. The susceptibility of the current *C. jejuni* host strains to commonly prescribed antibiotics (azithromycin, ciprofloxacin, nalidixic acid, clindamycin, and tetracycline) was also confirmed.

The *C. jejuni* host strains used in monophage production are not known to produce any functional enterotoxins (72) that could compromise the safety of the final product. While certain *C. jejuni* strains are known to produce enterotoxins, the host strains do not encode functional CDT.

The only production host strain-related toxin that is relevant for CampyShield safety is endotoxin or LPS. CampyShield phages are propagated in *C. jejuni* host strains. As with all Gram-negative bacteria, the outer membrane of *Campylobacter* contains lipopolysaccharide or LPS (73). Due to the lysis of host cells during the fermentation process (as the result of phage lytic cycle), *Campylobacter* LPS is present in the resulting phage lysates. Most of the endotoxin is expected to be removed during phage purification process.

LPS is of concern if sufficiently high amounts enter the human bloodstream, where it can trigger the signaling cascade for macrophage/endothelial cells to secrete pro-inflammatory cytokines and nitric oxide that may lead to "endotoxic shock." However, LPS has not been shown to cross the intestinal mucosa and oral administration of LPS shows no negative effects and may even elicit beneficial responses in the GI system (74). Additionally, there is no FDA specification for levels of endotoxin in oral products. Still, as a standard quality control protocol, Intralytix analyzes every CampyShield batch for the presence and levels of the LPS endotoxin in the final product. All product lots must be at or below 25,000 endotoxin unit (EU)/mL at ca. 9.0 $\log_{10}$ PFU/mL level in order to pass the release criteria for LPS. This level is very safe and is based upon the levels of endotoxins that are found naturally in healthy human saliva (1). See Section 3.1.2.3 for discussion of dietary intake.

6.2.1.3 MONOPHAGES

The safety of monophages is discussed in Section 6.1.1.2.

6.2.1.4 ANTIFOAM

Small amounts of the antifoam may be used in the initial fermentation of the individual monophages. Defoaming agents are listed in 21 CFR § 173.340.

6.2.2 QUALITY CONTROL

6.2.2.1 MONOPHAGES

The following tests are performed upon each monophage lot:

Lytic titer

The lytic titer test measures the lytic titer of each monophage lot, by determining the number of plaque forming units per milliliter (PFU/mL). The specification for each monophage lot is that the titer is ≥10.0 $\log_{10}$ PFU/mL. Lots failing to meet the specification due to a low titer may be appropriately concentrated and retested.

Microbial purity

The microbial purity test confirms that the monophage solution does not contain viable microbes. Briefly, samples of each monophage solution are tested by a) direct plating onto non-selective agar and b) plating after enrichment. The specification is that each monophage lot must be bacteriologically sterile. Lots failing the test may be re-filtered and retested. Lots repeatedly failing to meet the specification will be discarded.

Identity

Currently, genomic fingerprinting is used to confirm the identity of each monophage lot. The specification is that the sequence data matches the reference phage. Lots repeatedly failing the test will be discarded.

6.2.2.2 CAMPYSHIELD

The following tests are performed upon each batch of CampyShield:

Lytic titer test

The lytic titer test method confirms the titer (PFU/mL) of the CampyShield preparation. The specification for this test is CampyShield has a lytic titer of ≥10.0 $\log_{10}$ PFU/mL. Lots failing to meet the specification due to a low titer may be appropriately concentrated and retested.

Microbial purity

The microbial purity test is a determination of the viable microbial contamination in a phage solution. Briefly, a 1% representative sample of each lot of CampyShield is tested by combining with a concentrated growth medium and incubating for 14 days. Growth is monitored visually and by plating, if growth is not visually detectable. Both positive and negative controls are included. The specification for this test is that CampyShield must be bacteriologically sterile. Lots failing the test may be re-filtered and retested. Lots repeatedly failing to meet the specification will be discarded.

Endotoxin content test

Endotoxins are toxins associated with host bacteria, of which a residual amount could be present in the phage preparations. A commercially available quantitative Limulus Amebocyte Lysate (LAL)-based test specifically for measurement of endotoxin is currently used by Intralytix. The specification for this test is each lot of CampyShield must contain ≤ 25,000 EU/mL (at standard working concentration ca. 9.0 $\log_{10}$ PFU/mL). Lots failing to meet the specification may be washed with sterile PBS and subjected to the full panel of quality control tests.

Identity test

The identity test verifies that all phages claimed to be present in CampyShield are actually present. This method is a visual, signature-based confirmation that all monophages were included in the CampyShield lot during manufacturing. Briefly, as the lot is mixed, a second employee must be present to observe and confirm that each and every component monophage

is actually added. At least two employees must sign the preparation mixing worksheet, which is archived by the QC department for a minimum of 2 years.

6.3 SUBSTANTIAL EQUIVALENCE TO APPROVED PRODUCTS

6.3.1 PREVIOUSLY APPROVED BACTERIOPHAGE PREPARATIONS

Several lytic bacteriophage products targeting various bacterial pathogens have already been designated GRAS and/or cleared for food safety usage and other applications by a number of regulatory agencies:

- ListShield™ (formerly known as LMP-102,) a phage preparation containing six lytic *Listeria monocytogenes*-specific phages, is FDA-cleared as a food additive (21 CFR §172.785).
- ListShield is also GRAS (GRN 528).
- ListShield is also listed by the FSIS for use on various RTE meats and poultry products (FSIS Directive 7120.1).
- ListShield is also EPA-registered for use on non-food surfaces in food processing plants to prevent or significantly reduce contamination of *Listeria monocytogenes* (EPA registration #74234-1).
- Listex[2], a phage preparation containing a single *Listeria monocytogenes* lytic phage, P100, is GRAS (GRN 218).
- Listex is also listed by the FSIS for use as processing aid when applied at a level of $1x10^7$ to $1x10^9$ PFU/g food product (FSIS Directive 7120.1).
- SalmoFresh™, a phage preparation containing six *Salmonella*-specific lytic phages is GRAS (GRN 435)
- SalmoFresh is also listed by the FSIS for use on various poultry products (FSIS Directive 7120.1).
- Salmonelex™[3], a phage preparation containing two *Salmonella*-specific phages is GRAS (GRN 468).

[2] Currently marketed as PhageGuard L.

[3] Currently marketed as PhageGuard S.

- SalmPro® a phage preparation containing two *Salmonella*-specific phages is GRAS (GRN 752).
- EcoShield™ (formerly ECP-100) a phage preparation containing three lytic *E. coli* O157:H7-specific phages, is FDA-cleared for use as a food contact substance (FCN No. 1018).
- EcoShield is also listed by the FSIS for use as processing aid on red meat parts and trim prior to grinding (FSIS Directive 7120.1).
- EcoShield PX, a phage preparation containing three to eight lytic phages specific to shiga-toxin producing *E. coli*, is affirmed as GRAS (GRN 834).
- PhageGuard E™, a phage preparation containing two *E. coli*-specific lytic phages is GRAS (GRN 757).
- An *E. coli*-specific phage preparation containing six of 12 *E. coli*-specific bacteriophages is GRAS (GRN 724).
- ShigaShield™, a phage preparation containing five lytic phages to *Shigella* spp. is affirmed as GRAS (GRN 672).
- AgriPhage, a phage preparation targeting *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. Tomato, is EPA-registered for use on tomatoes and peppers (EPA Reg. No. 67986-1).
- Two bacteriophage preparations – one *Salmonella*-targeting and one *E. coli* O157:H7-targeting – are listed by the FSIS for use as processing aids on the hides and feathers of live animals before slaughter (FSIS Directive 7120.1).

Several regulatory agencies are represented in the preceding list, each of which separately concluded that a different bacteriophage preparation was safe and effective. The variety of these previously cleared or registered bacteriophage preparations attests to the general safety of bacteriophages and therefore supports their natural GRAS status. CampyShield is substantially equivalent to the above bacteriophage preparations and therefore is also GRAS.

6.4 SUMMARY AND BASIS FOR GRAS

CampyShield is an all-natural product made of three to eight *Campylobacter jejuni* specific lytic bacteriophages. All phages included in CampyShield are lytic phages and they have been rigorously characterized (including full genome sequencing) prior to inclusion in the cocktail.

Phages are omnipresent in the environment. Bacteriophages are the oldest, most ubiquitous organisms on earth, with their numbers estimated to be between 10^{30} and 10^{32}. Phages are present everywhere – including in our mouths, on our skin, and within our gastrointestinal tracks. They are also common and natural ingredients of all fresh, unprocessed foods. The

omnipresence of phages (including in foods) and their daily consumption by humans makes them naturally GRAS.

In further recognition of their safety, several lytic bacteriophage products targeting various bacterial pathogens have already been designated GRAS and/or cleared for food safety usage and other applications by a number of regulatory agencies.

Although all lytic bacteriophages are, by nature, GRAS, the phages in CampyShield must be verified to be lytic and to not contain any undesirable genes listed in 40 CFR § 725.421. The genomes of the three bacteriophages in CampyShield have been sequenced. Bioinformatic analysis of the component phages' sequences shows none contain any undesirable genes listed in 40 CFR §725.421. Furthermore, no antibiotic resistance gene, no 16S rRNA sequences, or other known toxin genes were identified in any of the phage genomes.

CampyShield is manufactured using Intralytix's standard procedures. These procedures have been reviewed by the FDA for manufacturing of Intralytix's bacteriophage food safety products, ListShield (21 CFR §172.785), EcoShield (FCN No. 1018), EcoShield PX (GRN 834), SalmoFresh (GRN 435), and ShigaShield (GRN 672) and are currently used to manufacture commercial lots of these products.

The only manufacturing byproduct of potential concern during CampyShield manufacturing is LPS. Intralytix tests every lot of CampyShield for LPS to ensure it meets the release criteria. The LPS levels of the CampyShield (at the working concentration of ca. $1x10^9$ PFU/mL) must be below 25,000 EU/mL for the lot to be released. This standard is the same as the maximum LPS level previously cleared by the FDA for EcoShield (per FCN 1018), SalmoFresh (GRN 435), and ShigaShield (GRN 672).

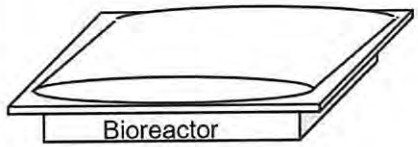
CampyShield is produced on animal product free media. The final CampyShield product contains no preservatives, known allergenic substances, or additives. CampyShield is eligible for certification as both Kosher and Halal, as the manufacturing process has previously been certified for both ListShield and SalmoFresh. These approvals will be pursued dependent upon market demands.

The proposed application rate for CampyShield is up to $1x10^8$ PFU per gram of food article. Assuming the maximum application rate of $1x10^8$ PFU/g to all foods within the target food groups, the average daily consumption of these foods would contain a mere 5.8 μg of phage particles, 38.1 mg of added sodium, and 1.4 mg of added potassium. This consumption would be spread out across several servings and meals, so the added sodium and potassium levels per serving would be so low as to not require any changes to labeling. The weight of added phage is negligible.

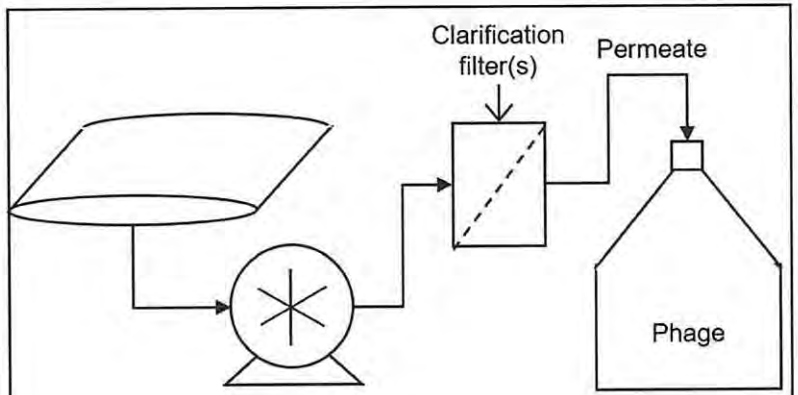
CampyShield is substantially equivalent to the lytic bacteriophage preparations that have been previously designated GRAS and/or cleared by other regulatory agencies. Furthermore, with the proposed maximum application rate for CampyShield of up to $1x10^8$ PFU per gram of food article, even in the worst case scenario ($1x10^8$ PFU/g) the rate is equal to or lower than the rates previously cleared for those other preparations as safe and effective. For instance, the maximum proposed application rate of CampyShield is 10 times lower than that of the previously GRAS-listed Listex P100 bacteriophage preparation.

In summary, the data presented in this document fully supports our designation of CampyShield as GRAS. The basis for our conclusion is five-fold. First, the scientific literature extensively documents that lytic bacteriophages pose no safety concerns to humans. Second, all bacteriophages in CampyShield are lytic, non-genetically modified, and free of any and all undesirable genes. Third, Intralytix's manufacturing process ensures the safety and quality of the final CampyShield product. Fourth, the estimated daily intake of the CampyShield phage preparation is so low it is negligible. And, fifth, the bacteriophage product is substantially equivalent to several bacteriophage products already receiving regulatory clearance. Based on this information, it is evident that CampyShield is GRAS.

Added to bioreactor:
-Growth media
-Bacterial host culture
-Monophage

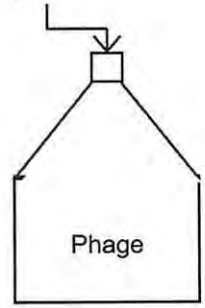


Inoculation, infection, and host lysis.

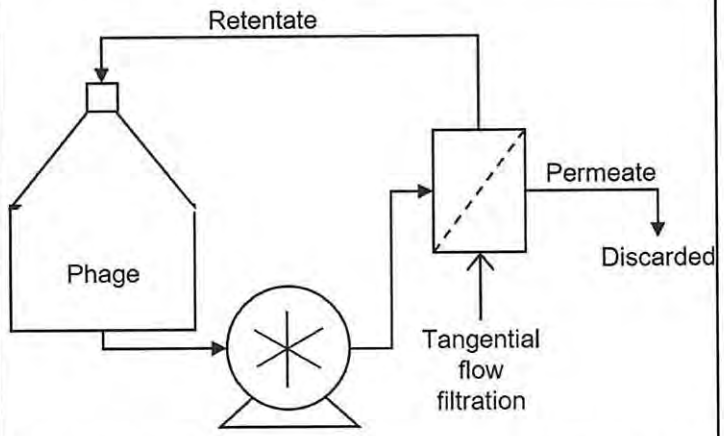


Clarification and removal of residual host bacteria. Permeate contains phage.

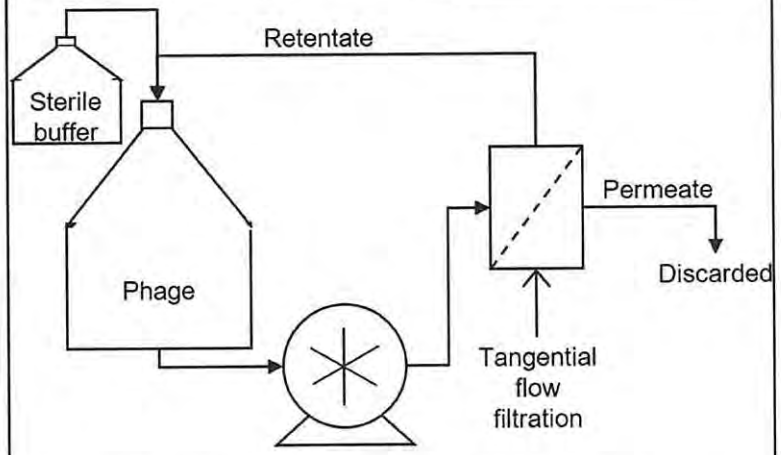
Added to vessel:
•DNase
•RNase



DNase and RNase treatment of phage permeate.



Concentration of phage ≥10:1 volume reduction. Retentate flow contains the monophage. Permeate flow contains smaller moieties.



Washing/Buffer Exchange. Retentate flow contains the monophage. Permeate contains smaller moieties. Ca. 10 to 1 volume exchange with sterile buffer.

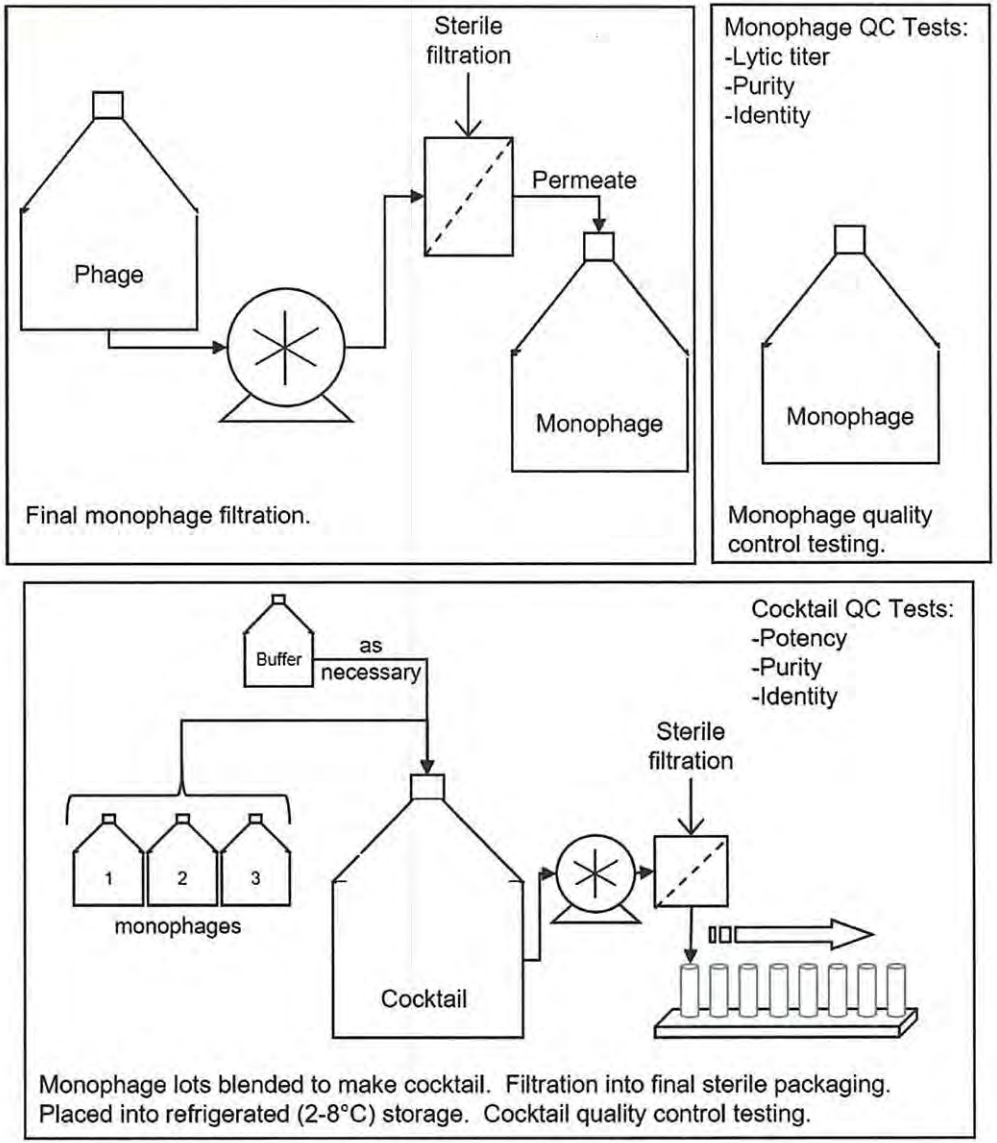


Figure 1 Overview of CampyShield™ manufacturing process

7 LIST OF SUPPORTING DATA AND INFORMATION

7.1 APPENDICES (INCLUDES NOT GENERALLY AVAILABLE DATA)

Appendix 1: Efficacy of CampyShield™ on Foods

7.2 REFERENCES (FOR GENERALLY AVAILABLE DATA)

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APPENDIX 1: EFFICACY STUDIES

Substance: Bacteriophage preparation (*Campylobacter jejuni* targeted)

Product:

Red meat including whole carcasses, primals, subprimals, cuts, trimmings, and organs

Raw poultry including carcasses, parts, and ground

Amount: Applied to the food product at a level of ca. ≤ $1x10^8$ plaque forming units (PFU) per gram of product

Reference: Acceptability determination

Labeling Requirements: None under the accepted conditions of use

CampyShield™ is an all-natural product comprised of *C. jejuni*-specific lytic bacteriophages. All phages included in CampyShield™ are lytic phages that have not been genetically manipulated in any way. The component phages of CampyShield™ are rigorously characterized prior to inclusion in the product.

The CampyShield™ preparation is intended for use in food products to control *C. jejuni* when added at ≤ $1x10^8$ PFU per gram of food. Intralytix, Inc. has concluded that CampyShield™ is generally recognized as safe (GRAS), and therefore, we believe it is not subject to the requirement of pre-market approval, under the conditions of its intended use.

CAMPYSHIELD™ IS EFFECTIVE.

Target range

CampyShield™ has been screened for its lytic activity against 61 *C. jejuni* trains. At the standard "working concentration" of $1x10^9$ PFU/mL, it lyses 67% of the *C. jejuni* strains in our collection.

Effect on *C. jejuni* levels in foods

CampyShield™ is intended to produce a statistically significant reduction of *C. jejuni* contamination vs. a control when applied as directed to food products.

Efficacy study summary

CampyShield™ was examined for its ability to reduce *C. jejuni* contamination when applied to various foods. Detailed reports of the studies are included in Appendix 1.1 - Appendix 1.4. A summary of the results is given below.

Description of the test system

For each food tested, portions were inoculated with *C. jejuni* CJ160 (ATCC 33292) isolate. After allowing the bacteria to colonize, the food was then treated with CampyShield™ or a control. The CampyShield™ contact times were 6 h, 24 h, and 48 h at 10°C, after which the *C. jejuni* was enumerated from the samples.

Summary of results

Red meat

Study CS20G20MCA examined the efficacy of CampyShield™ in reducing *C. jejuni* levels on veal loin chops. The CampyShield™ application rate was ca. $5x10^7$ PFU/g. CampyShield™ reduced the *C. jejuni* population by ca. 85% after 6h and 24h, and by 84% after 48h. The complete details of this study can be seen in Appendix 1.1.

Whole poultry

Study CS20G01MC examined the efficacy of CampyShield™ in reducing *C. jejuni* levels on chicken breast. The CampyShield™ application rate was ca. $7x10^7$ PFU/g. CampyShield™ reduced the *C. jejuni* population by ca. 84% after 6h, 87% after 24h, and 88% after 48h. The complete details of this study can be seen in Appendix 1.2.

Ground poultry

Study CS20G20MCB and Study CS20G22MC examined the efficacy of CampyShield™ in reducing *C. jejuni* levels on ground chicken. In both studies, the CampyShield™ application rate was ca. $7x10^7$ PFU/g and the effect after 6h, 24h, and 48h was examined. At each time point, CampyShield™ significantly reduced the *C. jejuni* population. In CS20G20MCB, the reductions were ca. 68%, 71%, and 67%, respectively. In CS20G22MC, the reductions were ca. 79%, 79%, and 72%, respectively.

Study CS20G22MC also examined whether CampyShield™ provides a continued technical effect by protecting the ground chicken against recontamination. When the ground chicken was recontaminated after treatment, there was no difference in the *C. jejuni* populations recovered from the samples previously treated with CampyShield™ or the control, indicating CampyShield™ does not provide a continued technical effect.

The complete details of these studies can be seen in Appendix 1.3 and 1.4.

Summary

We believe the data summarized here fully supports our conclusion that CampyShield™ is GRAS and our request for CampyShield™ to be included in FSIS directive 7120.1 as a safe and suitable ingredient used in the production of red meat and poultry products as a processing aid. Its intended use is as a spray applied to significantly reduce levels of *C. jejuni* when applied at $\leq 1x10^8$ PFU/g. Additionally, no foods treated to product specifications should require CampyShield™ as a listed ingredient on product labels.

Appendices

Appendix 1.1 Report CS20G20MCA

Veal loin chops

Appendix 1.2 Report CS20G01MC

Chicken breast

Appendix 1.3 Report CS20G20MCB

Ground chicken

Appendix 1.4 Report CS20G22MC

Ground chicken -Recontamination

intralytix
SAFETY BY NATURE

Appendix 1.1:
Study
#CS20G20MCA

Evaluation of the ability of CampyShield™ to reduce *Campylobacter* on experimentally contaminated veal loin chops

Study # CS20G20MCA

Intralytix
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1 STUDY TITLE

Evaluation of the ability of CampyShield™ to reduce *Campylobacter* on experimentally contaminated veal loin chops

2 STUDY DIRECTOR

Amit Vikram, Ph.D.

3 STUDY PERSONNEL

Name:	Title:	Role:
Mary Theresa Callahan, MS	Research Scientist	Hands-on-research / Data Review / Report Assembly
Amit Vikram, Ph.D.	Senior Research Scientist	Study Director / Data Review / Report Assembly
Joelle Woolston, MS	Director, Laboratory Operations	Data review / Report Assembly

4 PERFORMING LABORATORY

Intralytix, Inc.
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5 STUDY OBJECTIVE

To determine the effectiveness of CampyShield™ in reducing the numbers of viable *Campylobacter jejuni* on veal loin chops when applied at the rate of 4 mL per lb of veal.

6 TEST MATRIX

Fresh veal loin chops were purchased on the day of the experiment at a Columbia, MD area supermarket. The veal chops were not washed or pre-treated prior to our studies.

7 CAMPYSHIELD™ LOT AND APPLICATION RATE

- CampyShield™ Lot #0420G2710testB
- Titer: $6x10^9$ PFU/mL
- The application rate was ca. 4 mL CampyShield™ per pound of veal.
- CampyShield™ was applied using Burkle 20 mL spray bottles with pump vaporizer (Burkle Inc, Bohemia, NY)

8 BACTERIAL STRAIN USED TO EXPERIMENTALLY CONTAMINATE VEAL

The veal test matrix was experimentally contaminated with a single *Campylobacter* strain:

- *CJ160: Campylobacter jejuni* subsp. *jejuni* strain ATCC33292, which was isolated from human feces

Shortly before performing the study, the strain was thawed and grown (42 ± 2°C; 10% CO_2, 5% O_2, 85% N_2) in cation-adjusted brain heart infusion (cBHI) broth for 24 h, which corresponds to ca. $7x10^9$ CFU/mL. The culture was diluted 1000-fold in phosphate buffered saline (PBS) prior to application to veal.

The veal was experimentally contaminated by ca. $1x10^4$ CFU of *Campylobacter* / g of veal.

9 MEDIA AND REAGENTS

- BHI broth (Sigma-Aldrich, Burlington, MA; catalog # 53286-500G)
- $CaCl_2$ (Sigma-Aldrich, Burlington, MA; catalog # C-3306)
- $MgSO_4$ (Fisher Chemicals, Hampton, NH; catalog # M65-500)
- Campy CVA agar (Becton, Dickinson and Co., Sparks, MD; catalog # 297246)
- Peptone water (Becton, Dickinson and Co., Sparks, MD; catalog # 212367)
- PBS (pH 7.4) (Life Technologies, Grand Island, NY; catalog # 10010031)

10 GENERAL OUTLINE OF STUDY

1. Veal samples (25 g each; n=19) were cut from the bone, weighed and placed in sterile containers with lids.

2. The challenge dose of bacteria was applied onto the veal surfaces via pipette and spread over the veal surface using a cell spreader. One sample was not treated with the bacterial culture as the uncontaminated, untreated control.

3. The bacteria were allowed to colonize the veal matrix at room temperature (approximately 22°C) for 20 min.

4. cBHI (control) or CampyShield™ was applied as described in Section 7. Samples were sprayed with 0.22 mL of either cBHI (control) or CampyShield™. The final concentration of CampyShield™ was ca. 5.3 x 10^7 PFU/g.

5. Following treatment, the container lids were closed and the samples were incubated at 10°C for up to 48 h.

6. At 6 h, 24 h, and 48 h post-treatment, triplicate samples from each treatment group of were removed, placed into sterile filter bags, and 50 mL of sterile peptone water was added. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

7. The number of viable *Campylobacter* was determined by plating 0.1 mL aliquots of the stomached meat/peptone water mixture and a 1:10 dilution (in PBS) onto separate Campy CVA agar plates. The plates were incubated (42°C, 10% CO_2, 5% O_2, 85% N_2) for 48 h and the CFU/g of sample were calculated after counting the colonies, as follows:

$$\frac{CFU\ recovered}{g\ of\ treated\ poultry} = \frac{CFU}{0.1\ mL\ plated} \times \frac{50\ mL\ peptone\ water}{25\ g\ sample}\ (\div\ dilution)$$

11 RESULTS

11.1 RAW DATA

Table 1 Raw Data for Study #CS20G20MCA

Treatment	Treatment time (h)	Bacterial challenge (CFU/g)	Number of replicates	CFU/g recovered
Control	6	Yes	3	6000, 4800, 5600
~5x10^7 PFU/g CampyShield	6	Yes	3	520, 600, 1280
Control	24	Yes	3	3800, 3800, 2200
~5x10^7 PFU/g CampyShield	24	Yes	3	540, 280, 640
Control	48	Yes	3	1600, 1900, 1120
~5x10^7 PFU/g CampyShield	48	Yes	3	280, 240, 220
Untreated Control	NA	No	1	0

11.2 TABULAR PRESENTATION OF RESULTS

Table 2 Tabular Summary of the Results for Study #CS20G20MCA

Treatment	Treatment Time	Total # replicates	Mean CFU/g	Average Percent reduction	Average Log reduction	Significance at p<0.05
Control	6 h	3	5467	NA	NA	
~5x10^7 PFU/g CampyShield	6 h	3	800	85%	0.83	Yes (p<0.001)
Control	24 h	3	3267	NA	NA	
~5x10^7 PFU/g CampyShield	24 h	3	487	85%	0.83	Yes (p<0.001)
Control	48 h	3	1540	NA	NA	
~5x10^7 PFU/g CampyShield	48 h	3	247	84%	0.80	Yes (p<0.001)

11.3 GRAPHICAL PRESENTATION OF RESULTS

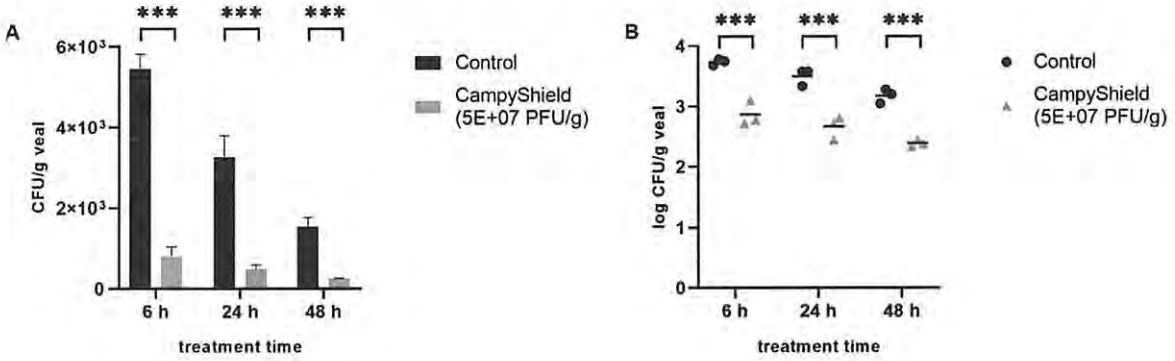

Figure 1 **Overall reduction in *Campylobacter* levels on veal loin chops by CampyShield™ (~5x10^7 PFU/g) compared to control after 6 h, 24 h, and 48 h**

A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. (* = p<0.001).**

11.4 STATISTICAL ANALYSIS

The efficacy of the CampyShield™ treatment in reducing the number of viable *Campylobacter* in the experimentally contaminated veal was evaluated by comparing the data obtained from control samples with the CampyShield™-treated samples.

Statistical analysis was performed using GraphPad Prism for Windows (version 8.0.2; GraphPad Software; San Diego, CA; www.graphpad.com). The data was analyzed using Two-Way Analysis of Variance (Two-Way ANOVA). Sidak's Multiple Comparison test was performed to compare the treatment effect at 6 h, 24 h, and 48 h treatment times. A P value <0.05 was considered statistically significant.

ANOVA

H_0: The sample mean of *Campylobacter* populations enumerated from Control and CampyShield™ treatments are the same.

H_1: The sample mean of *Campylobacter* populations enumerated from Control and CampyShield™ treatments are different.

P value

The P value is <0.001, considered highly significant (H_0 rejected; H_1 accepted).

In summary, the mean populations of *Campylobacter* recovered from veal are significantly different between the Control and CampyShield™ treatments.

The effect of CampyShield™ over treatment duration (i.e., 6 h vs. 24 h vs. 48 h) was determined using a two-way ANOVA to analyze the interaction between the two variables (treatment and time).

ANOVA

H_0: The average reduction in *Campylobacter* populations following CampyShield™ treatment is equal at all timepoints.

H_1: The average reduction in *Campylobacter* populations following CampyShield™ treatment is different at various timepoints.

P value

The P value is 0.88, considered not significant (H_0 accepted).

In summary, increasing treatment time does not significantly increase the efficacy of CampyShield™ at reducing *Campylobacter* on veal.

11.5 BRIEF DISCUSSION OF RESULTS AND STUDY'S CONCLUSIONS

- Applying $6x10^9$ PFU/mL CampyShield™ to veal – at the rate of 4.0 mL per lb of veal - reduced the number of viable *Campylobacter* by ca. 85% after 6 h, 85% after 24 h, and 84% after 48 h of incubation at 10°C. The observed reduction was statistically significant ($P<0.001$).

- Increasing CampyShield™ treatment time from 6 h to 24 h or 48 h did not significantly affect the observed reduction of *Campylobacter* on veal ($P>0.05$).

12 SUMMARY CONCLUSION OF THE STUDY

CampyShield™ significantly reduced the *Campylobacter* population on veal samples by ca. 85% when it was applied to the experimentally contaminated meat.

Increasing the treatment time beyond 6 h did not increase the efficacy of CampyShield™ against *Campylobacter*, indicating there was no continued technical effect of CampyShield™ on the food.

13 SIGNATURES

[redacted]

Mary Theresa Callahan, MS
Research Scientist
Hands on Research

14th August 2020
Date

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Amit Vikram, PhD
Senior Research Scientist
Study Director

14th August 2020
Date

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Joelle Woolston, MS
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Date

Appendix 1.2
Study
#CS20G01MC

Evaluation of the ability of CampyShield™ to reduce *Campylobacter* on experimentally contaminated chicken breasts

Study # CS20G01MC

Intralytix
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1 STUDY TITLE

Evaluation of the ability of CampyShield™ to reduce *Campylobacter* on experimentally contaminated chicken breasts.

2 STUDY DIRECTOR

Amit Vikram, Ph.D.

3 STUDY PERSONNEL

Name:	Title:	Role:
Mary Theresa Callahan, MS	Research Scientist	Hands-on-research / Data Review / Report Assembly
Amit Vikram, Ph.D.	Senior Research Scientist	Study Director / Data Review / Report Assembly
Joelle Woolston, MS	Director, Laboratory Operations	Data review / Report Assembly

4 PERFORMING LABORATORY

Intralytix, Inc.
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5 STUDY OBJECTIVE

To determine the effectiveness of CampyShield™ in reducing the numbers of viable *Campylobacter jejuni* on chicken breast when applied at the rate of 4 mL per lb of poultry.

6 TEST MATRIX

Fresh chicken breast tenderloins were purchased on the day of the experiment at a Columbia, MD area supermarket. The tenderloins were not washed or pre-treated prior to our studies.

7 CAMPYSHIELD™ LOT AND APPLICATION RATE

- CampyShield™ Lot #0420F3010testA
- Titer: $7.6x10^9$ PFU/mL
- The application rate was ca. 4 mL CampyShield™ per pound of poultry.
- CampyShield™ was applied using Burkle 20 mL spray bottles with pump vaporizer (Burkle Inc, Bohemia, NY)

8 BACTERIAL STRAIN USED TO EXPERIMENTALLY CONTAMINATE POULTRY

The poultry test matrix was experimentally contaminated with a single *Campylobacter* strain:

- *CJ160: Campylobacter jejuni* subsp. *jejuni* strain ATCC33292, which was isolated from human feces

Shortly before performing the study, the strain was thawed and grown in cation-adjusted brain heart infusion (cBHI) broth at 42 (± 2) °C under microaerophilic conditions (10% CO_2, 5% O_2, 85% N_2) for 24 h, which corresponds to ca. $7x10^9$ CFU/mL. The culture was diluted 1000-fold in phosphate buffered saline (PBS) prior to application to chicken breast.

The chicken was experimentally contaminated by ca. $1x10^4$ CFU of *Campylobacter* / g of chicken breast.

9 MEDIA AND REAGENTS

- BHI broth (Sigma-Aldrich, Burlington, MA; catalog # 53286-500G)
- $CaCl_2$ (Sigma-Aldrich, Burlington, MA; catalog # C-3306)
- $MgSO_4$ (Fisher Chemicals, Hampton, NH; catalog # M65-500)
- Campy CVA agar (Becton, Dickinson and Co., Sparks, MD; catalog # 297246)
- Peptone water (Becton, Dickinson and Co., Sparks, MD; catalog # 212367)
- PBS (pH 7.4) (Life Technologies, Grand Island, NY; catalog # 10010031)

10 GENERAL OUTLINE OF STUDY

1. Chicken breast samples (50-g each; n=19) were weighed and placed in sterile lidded containers.

2. The challenge dose of bacteria was applied onto the chicken breast surfaces via pipette and spread over the chicken breast samples using a cell spreader. One sample was not treated with the bacterial culture and served as the uncontaminated, untreated control.

3. The bacteria were allowed to colonize the chicken breast matrix at room temperature (approximately 22°C) for 20 min.

4. PBS (control) or CampyShield™ was applied as described in Section 7. Samples were sprayed with 0.44 mL of either PBS (control) or CampyShield™. The final concentration of CampyShield™ was ca. 6.7×10^7 PFU/g.

5. Following treatment, the container lids were closed and the samples were incubated at 10°C for up to 48 h.

6. At 6 h, 24 h, and 48 h post-treatment, triplicate samples from each treatment group of were removed, placed into sterile filter bags, and 100 mL of sterile peptone water was added. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

7. The viable *Campylobacter* was enumerated by plating 0.1 mL aliquots of the stomached meat/peptone water mixture and a 1:10 dilution (in PBS) onto separate Campy CVA agar plates. The plates were incubated (42°C, 10% CO_2, 5% O_2, 85% N_2) for 48 h and colonies were counted. The CFU/g of sample were calculated as follows:

$$\frac{CFU\ recovered}{g\ of\ treated\ poultry} = \frac{CFU}{0.1\ mL\ plated} \times \frac{100\ mL\ peptone\ water}{50\ g\ sample}\ (\div dilution)$$

11 RESULTS

11.1 RAW DATA

Table 1 Raw Data for Study #CS20G01MC

Treatment	Treatment time (h)	Bacterial challenge (CFU/g)	Number of replicates	CFU/g recovered
Control	6	Yes	3	5000, 6200, 4600
~7x10^7 PFU/g CampyShield™	6	Yes	3	1020, 660, 780
Control	24	Yes	3	6600, 5600, 5400
~7x10^7 PFU/g CampyShield™	24	Yes	3	600, 800, 690
Control	48	Yes	3	1600, 1400, 2320
~7x10^7 PFU/g CampyShield™	48	Yes	3	120, 200, 300
Uninoculated/Untreated Control	NA	No	1	0

11.2 TABULAR PRESENTATION OF RESULTS

Table 2 Tabular Summary of the Results for Study #CS20G01MC

Treatment	Treatment Time	Total # replicates	Mean CFU/g	Average Percent reduction	Average Log reduction	Significance at p<0.05
Control	6 h	3	5267	NA	NA	
~7x10^7 PFU/g CampyShield™	6 h	3	820	84%	0.81	Yes (p<0.001)
Control	24 h	3	5867	NA	NA	
~7x10^7 PFU/g CampyShield™	24 h	3	787	87%	0.87	Yes (p<0.001)
Control	48 h	3	1773	NA	NA	
~7x10^7 PFU/g CampyShield™	48 h	3	207	88%	0.93	Yes (p<0.001)

11.3 GRAPHICAL PRESENTATION OF RESULTS

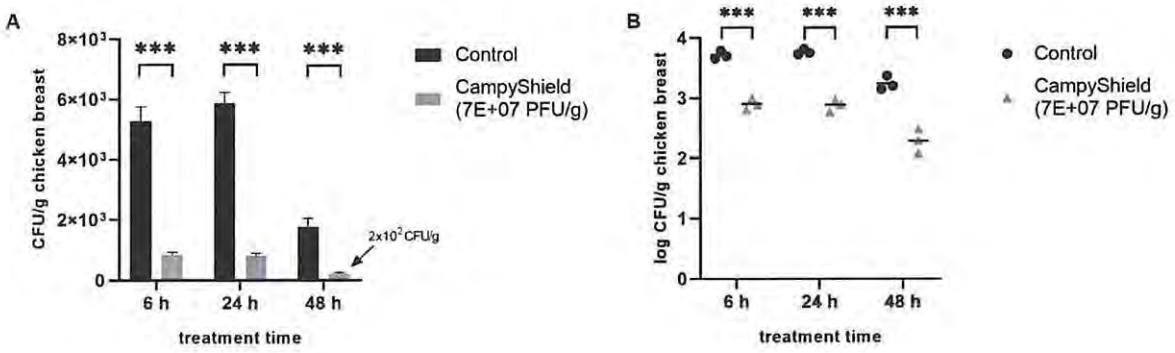

Figure 1 **Overall reduction in *Campylobacter* levels on chicken breast by CampyShield™ (~7x10^7 PFU/g) compared to control after 6 h, 24 h, and 48 h**

A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. (* = p<0.001).**

11.4 STATISTICAL ANALYSIS

The efficacy of the CampyShield™ treatment in reducing the number of viable *Campylobacter* in the experimentally contaminated chicken breast was evaluated by comparing the data obtained from control samples with the CampyShield™-treated samples.

Statistical analysis was performed using GraphPad Prism for Windows (version 8.0.2; GraphPad Software; San Diego, CA; www.graphpad.com). The data was analyzed using Two-Way Analysis of Variance (Two-Way ANOVA). Sidak's Multiple Comparison test was performed to compare the treatment effect at 6 h, 24 h, and 48 h treatment times. A P value <0.05 was considered statistically significant.

ANOVA

H_0: The sample means of *Campylobacter* recovered from Control and CampyShield™ treatments are equal.

H_1: The sample means of *Campylobacter* recovered from Control and CampyShield™ treatments are different.

P value

The P value is <0.001, considered highly significant (H_0 rejected; H_1 accepted).

In summary, the mean populations of *Campylobacter* recovered from poultry are significantly different between the Control and CampyShield™ treatments.

The effect of CampyShield™ over treatment duration (i.e., 6 h vs. 24 h vs. 48 h) was determined using a two-way ANOVA to analyze the interaction between the two variables (treatment and time).

ANOVA

H_0: The average reduction in *Campylobacter* populations following CampyShield™ treatment is equal at all timepoints.

H_1: The average reduction in *Campylobacter* populations following CampyShield™ treatment is different at various timepoints.

P value

The P value is 0.57, considered not significant (H_0 accepted).

In summary, increasing treatment time does not significantly increase the efficacy of CampyShield™ at reducing *Campylobacter* on chicken.

11.5 BRIEF DISCUSSION OF RESULTS AND STUDY'S CONCLUSIONS

- Applying $7x10^9$ PFU/mL CampyShield™ to chicken breast – at the rate of 4.0 mL per lb of poultry - reduced the number of viable *Campylobacter* by ca. 84% after 6 h, 87% after 24 h, and 88% after 48 h of incubation at 10°C. The observed reductions were statistically significant ($P<0.001$).

- Increasing CampyShield™ treatment time from 6 h to 24 h or 48 h did not significantly affect the observed reduction of *Campylobacter* on chicken breast ($P>0.05$).

12 SUMMARY CONCLUSION OF THE STUDY

CampyShield™ significantly reduced the *Campylobacter* population on chicken breast samples by ca. 86% when it was applied to the experimentally contaminated poultry meat.

Increasing the treatment time beyond 6 h did not increase the efficacy of CampyShield™ against *Campylobacter*, indicating there was no continued technical effect of CampyShield™ on the food.

13 SIGNATURES

[redacted]

Mary Theresa Callahan, MS
Research Scientist
Hands on Research

14th August 2020
Date

[redacted]

Amit Vikram, PhD
Senior Research Scientist
Study Director

14th August 2020
Date

[redacted]

Joelle Woolston, MS
Director, Laboratory Operations

14-Aug-2020
Date

Appendix 1.3:
Study
#CS20G20MCB

Evaluation of the ability of CampyShield™ to reduce *Campylobacter* in experimentally contaminated ground chicken

Study # CS20G20MCB

Intralytix
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1 STUDY TITLE

Evaluation of the ability of CampyShield™ to reduce *Campylobacter jejuni* in experimentally contaminated ground chicken.

2 STUDY DIRECTOR

Amit Vikram, Ph.D.

3 STUDY PERSONNEL

Name:	Title:	Role:
Mary Theresa Callahan, MS	Research Scientist	Hands-on-research / Data Review / Report Assembly
Amit Vikram, Ph.D.	Senior Research Scientist	Study Director / Data Review / Report Assembly
Joelle Woolston, MS	Director, Laboratory Operations	Data review / Report Assembly

4 PERFORMING LABORATORY

Intralytix, Inc.
Research and Development
8681 Robert Fulton Dr
Columbia, MD 21046

5 STUDY OBJECTIVE

To determine the effectiveness of CampyShield™ in reducing the numbers of viable *Campylobacter jejuni* in ground chicken when applied at the rate of 4 mL per lb of poultry.

6 TEST MATRIX

Fresh ground chicken was purchased on the day of the experiment at a Columbia, MD area supermarket. The ground chicken was not pre-treated prior to our studies.

7 CAMPYSHIELD™ LOT AND APPLICATION RATE

- CampyShield™ Lot #0420F3010testA
- Titer: $7.6x10^9$ PFU/mL
- The application rate was ca. 4 mL CampyShield™ per pound of ground chicken
- CampyShield™ was applied using Burkle 20 mL spray bottles with pump vaporizer (Burkle Inc, Bohemia, NY)

8 BACTERIAL STRAIN USED TO EXPERIMENTALLY CONTAMINATE GROUND CHICKEN

The ground chicken test matrix was experimentally contaminated with a single *Campylobacter* strain:

- *CJ160: Campylobacter jejuni* subsp. *jejuni* strain ATCC33292, which was isolated from human feces

Shortly before performing the study, the strain was thawed and grown (42 ± 2°C; 10% CO_2, 5% O_2, 85% N_2) in cation-adjusted brain heart infusion (cBHI) broth for 24 h, which corresponds to ca. $7x10^9$ CFU/mL. The culture was diluted 1000-fold in phosphate buffered saline (PBS) prior to application to ground chicken.

The ground chicken was experimentally contaminated by ca. $1x10^4$ CFU of *Campylobacter* / g of ground chicken.

9 MEDIA AND REAGENTS

- BHI broth (Sigma-Aldrich, Burlington, MA; catalog # 53286-500G)
- $CaCl_2$ (Sigma-Aldrich, Burlington, MA; catalog # C-3306)
- $MgSO_4$ (Fisher Chemicals, Hampton, NH; catalog # M65-500)
- Campy CVA agar (Becton, Dickinson and Co., Sparks, MD; catalog # 297246)
- Peptone water (Becton, Dickinson and Co., Sparks, MD; catalog # 212367)
- PBS (pH 7.4) (Life Technologies, Grand Island, NY; catalog # 10010031)

10 GENERAL OUTLINE OF STUDY

1) Two 325-g samples of ground chicken were weighed into sterile mixing bowls. An additional 25-g sample for each experiment was not treated with bacterial cultures as the uncontaminated, untreated control.

2) The challenge dose of bacteria was applied onto the ground chicken surface via pipette and the bacteria were allowed to colonize the ground chicken matrix at room temperature (approximately 22°C) for 20 min. Then, samples were mixed at the lowest mixing speed ("stir") for 10 min using a KitchenAid stand mixer with the flat beater attachment.

3) PBS (control) or CampyShield™ was applied to the samples as described in Section 7. Samples were sprayed with 2.86 mL of either PBS (control) or CampyShield™. The final concentration of CampyShield™ was ca. 6.7×10^7 PFU/g.

4) Following treatment, samples sat for 10 min at room temperature, then were mixed for an additional 10 min.

5) After mixing, 25-g samples (n=9) were weighed from each treatment group into separate sterile filter bags then the bags were sealed and placed at 10°C. The remaining 100g of ground chicken was covered in plastic wrap and placed at 10°C.

6) At 6 h, 24 h, and 48 h post-treatment, triplicate sample bags from each treatment group were removed and 50 mL of sterile peptone water was added. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

7) The number of viable *Campylobacter* was determined by plating 0.1 mL aliquots of the stomached meat/peptone water mixture and a 1:10 dilution (in PBS) onto separate Campy CVA agar plates. The plates were incubated (42°C, 10% CO_2, 5% O_2, 85% N_2) for 48 h, and the CFU/g of sample were calculated after counting the colonies, as follows:

$$\frac{CFU\ recovered}{g\ of\ treated\ poultry} = \frac{CFU}{0.1\ mL\ plated} \times \frac{50\ mL\ peptone\ water}{25\ g\ sample}\ (\div dilution)$$

11 RESULTS

11.1 RAW DATA

Table 1: Raw Data for Study #CS20G20MCB

Treatment	Treatment time (h)	Bacterial challenge (CFU/g)	Number of replicates	CFU/g recovered
Control	6	Yes	3	28800, 28400, 30800
~7x10^7 PFU/g CampyShield™	6	Yes	3	6000, 11200, 11200
Control	24	Yes	3	10000, 11800, 12400
~7x10^7 PFU/g CampyShield™	24	Yes	3	3800, 3200, 3000
Control	48	Yes	3	8800, 10600, 8200
~7x10^7 PFU/g CampyShield™	48	Yes	3	3200, 3200, 2600
Untreated Control	NA	No	1	0

11.2 TABULAR PRESENTATION OF RESULTS

Table 2: Tabular Summary of the Results for Study #CS20G20MCB

Treatment	Treatment Time	Total # replicates	Mean CFU/g	Average Percent reduction	Average Log reduction	Significance at p<0.05
Control	6 h	3	29333	NA	NA	
~7x10^7 PFU/g CampyShield™	6 h	3	9467	68%	0.49	Yes (p<0.001)
Control	24 h	3	11400	NA	NA	
~7x10^7 PFU/g CampyShield™	24 h	3	3333	71%	0.53	Yes (p<0.001)
Control	48 h	3	9200	NA	NA	
~7x10^7 PFU/g CampyShield™	48 h	3	3000	67%	0.49	Yes (p<0.001)

11.3 GRAPHICAL PRESENTATION OF RESULTS

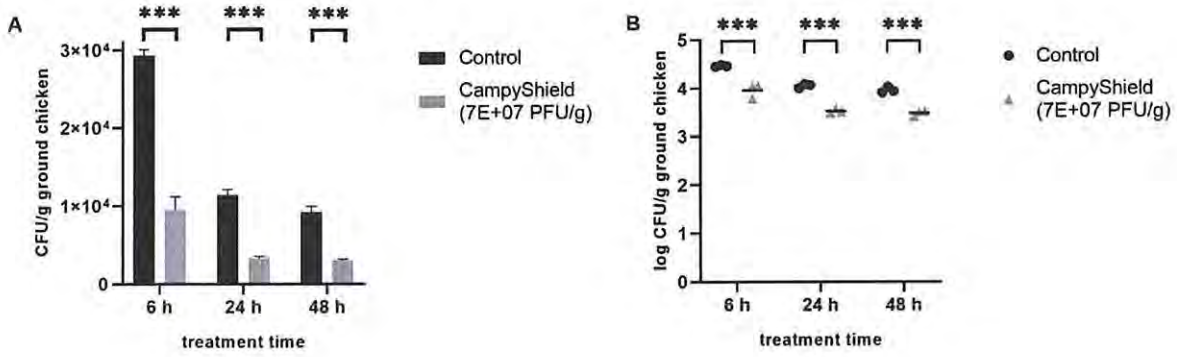

Figure 1 Overall reduction in Campylobacter levels on ground chicken by CampyShield™ (~7x10^7 PFU/g) compared to control after 6 h, 24 h, and 48 h. A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. (* = p<0.001).**

11.4 STATISTICAL ANALYSIS

The efficacy of the CampyShield™ treatment in reducing the number of viable *Campylobacter* in the experimentally contaminated ground chicken was evaluated by comparing the data obtained from control samples with the CampyShield™-treated samples.

Statistical analysis was performed using GraphPad Prism for Windows (version 8.0.2; GraphPad Software; San Diego, CA; www.graphpad.com). The data was analyzed using Two-Way Analysis of Variance (Two-Way ANOVA). Sidak's Multiple Comparison test was performed to compare the treatment effect at 6 h, 24 h, and 48 h treatment time. A P value <0.05 was considered statistically significant.

ANOVA

H_0: The mean numbers of *Campylobacter* recovered from Control and CampyShield™ treatments are the same.

H_1: The mean numbers of *Campylobacter* recovered from Control and CampyShield™ treatments are different.

P value

The P value is <0.001, considered highly significant (H_0 rejected; H_1 accepted).

In summary, the mean populations of *Campylobacter* recovered from ground chicken are significantly different between the Control and CampyShield™ treatments.

The effect of CampyShield™ over treatment duration (i.e., 6 h vs. 24 h vs. 48 h) was determined using a two-way ANOVA to analyze the interaction between the two variables (treatment and time).

ANOVA

H_0: The average reductions in Campylobacter populations following CampyShield™ treatment are same at all timepoints.

H_1: CampyShield™ does not have the same effect on *Campylobacter* populations at all timepoints.

P value

The P value is 0.90, considered not significant (H_0 accepted).

In summary, increasing treatment time does not significantly increase the efficacy of CampyShield™ at reducing *Campylobacter* in ground chicken.

11.5 BRIEF DISCUSSION OF RESULTS AND STUDY'S CONCLUSIONS

- Applying $7x10^9$ PFU/mL CampyShield™ to ground chicken – at the rate of 4.0 mL per lb of ground chicken – significantly reduced the number of viable *Campylobacter* by ca. 68% after 6 h, 71% after 24 h, and 67% after 48 h of incubation at 10°C. The observed reductions were statistically significant ($P<0.001$).
- Increasing CampyShield™ treatment time from 6 h to 24 h or 48 h did not significantly affect the observed reduction of *Campylobacter* in ground chicken ($P>0.05$).

12 SUMMARY CONCLUSION OF THE STUDY

CampyShield™ significantly reduced the *Campylobacter* population in ground chicken samples by ca. 69% when it was applied to the experimentally contaminated meat stored at 10°C.

Increasing the treatment time beyond 6 h did not increase the efficacy of CampyShield™ against *Campylobacter,* indicating there was no continued technical effect of CampyShield™ on the food.

13 SIGNATURES

[redacted]

Mary Theresa Callahan, MS
Research Scientist
Hands on Research

14th August 2020
Date

[redacted]

Amit Vikram, PhD
Senior Research Scientist
Study Director

14th August 2020
Date

[redacted]

Joelle Woolston, MS
Director, Laboratory Operations

14-Aug-2020
Date

Appendix 1.4:
Study
#CS20G22MC

Evaluation of the ability of CampyShield™ to (i) reduce *Campylobacter* contamination of ground chicken, and (ii) provide protection against recontamination of ground chicken with *Campylobacter*

Study # CS20G22MC

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1 STUDY TITLE

Evaluation of the ability of CampyShield™ to (i) reduce *Campylobacter* contamination of ground chicken and (ii) provide protection against recontamination of ground chicken with *Campylobacter*

2 STUDY DIRECTOR

Amit Vikram, Ph.D.

3 STUDY PERSONNEL

Name:	Title:	Role:
Mary Theresa Callahan, MS	Research Scientist	Hands-on-research / Data Review / Report Assembly
Amit Vikram, Ph.D.	Senior Research Scientist	Study Director / Data Review /Report Assembly
Joelle Woolston, MS	Director, Laboratory Operations	Data review / Report Assembly

4 PERFORMING LABORATORY

Intralytix, Inc.
Research and Development
8681 Robert Fulton Dr
Columbia, MD 21046

5 STUDY OBJECTIVE

To determine whether application of CampyShield™ (i) reduces the number of viable *Campylobacter jejuni* in experimentally contaminated ground chicken when applied at a rate of 4 mL per lb of poultry, and (ii) provides a residual technical effect against recontamination of ground chicken with *Campylobacter*.

6 TEST MATRIX

Fresh ground chicken was purchased on the day of the experiment at a Columbia, MD area supermarket. The ground chicken was not pre-treated prior to our studies.

7 CAMPYSHIELD™ LOT AND APPLICATION RATE

- CampyShield™ Lot #0420F3010testA
- Titer: $7.6x10^9$ PFU/mL
- The application rate was ca. 4 mL CampyShield™ per pound of ground chicken.
- CampyShield™ was applied using Burkle 20 mL spray bottles with pump vaporizer (Burkle Inc, Bohemia, NY)

8 BACTERIAL STRAIN USED TO EXPERIMENTALLY CONTAMINATE GROUND CHICKEN

The ground chicken test matrix was experimentally contaminated with a single *Campylobacter* strain:

- *CJ160: Campylobacter jejuni* subsp. *jejuni* strain ATCC33292, which was isolated from human feces

Shortly before performing the study, the strain was thawed and grown (42 ± 2°C; 10% CO_2, 5% O_2, 85% N_2) in cation-adjusted brain heart infusion (cBHI) broth for 24 h, which corresponds to ca. $7x10^9$ CFU/mL. The culture was diluted 1000-fold in phosphate buffered saline (PBS) prior to application to ground chicken.

The ground chicken was experimentally contaminated by ca. $1x10^4$ CFU of *Campylobacter* / g of ground chicken.

9 MEDIA AND REAGENTS

- BHI broth (Sigma-Aldrich, Burlington, MA; catalog # 53286-500G)
- $CaCl_2$ (Sigma-Aldrich, Burlington, MA; catalog # C-3306)
- $MgSO_4$ (Fisher Chemicals, Hampton, NH; catalog # M65-500)
- Campy CVA agar (Becton, Dickinson and Co., Sparks, MD; catalog # 297246)
- Peptone water (Becton, Dickinson and Co., Sparks, MD; catalog # 212367)
- PBS (pH 7.4) (Life Technologies, Grand Island, NY; catalog # 10010031)

10 GENERAL OUTLINE OF STUDY

1) Two 325-g samples of ground chicken were weighed into sterile mixing bowls. An additional 25-g sample for each experiment was not treated with bacterial cultures as the uncontaminated, untreated control.

2) The challenge dose of bacteria was applied onto the ground chicken surface via pipette and the bacteria were allowed to colonize the ground chicken matrix at room temperature (approximately 22°C) for 20 min. Then, samples were mixed at the lowest mixing speed ("stir") for 10 min using a KitchenAid™ stand mixer with the flat beater attachment.

3) PBS (control) or CampyShield™ was applied to the samples as described in Section 7. Samples were sprayed with 2.86 mL of either PBS (control) or CampyShield™. The final concentration of CampyShield™ was ca. $6.7x10^7$ PFU/g.

4) Following treatment, samples sat for 10 min at room temperature, then were mixed for an additional 10 min.

5) After mixing, 25 g samples (n=9) were weighed from each treatment group into separate sterile filter bags then the bags were sealed and placed at 10°C. The remaining 100 g of ground chicken was covered in plastic wrap and placed at 10°C.

6) At 6 h, 24 h, and 48 h post-treatment, triplicate samples bags from each treatment group were removed and 50 mL of sterile peptone water was added. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

7) At 48 h post-treatment, the 100 g samples from Step 5 (inoculated and treated ground chicken samples) were re-inoculated with 100 µL of a freshly prepared CJ160 inoculum, as described in Section 8. The resulting bacterial challenge was ca. $1x10^4$ CFU/g of ground chicken.

   - Following inoculation, samples were incubated at room temperature for 20 min, and then were mixed for an additional 10 min on the lowest mixing setting. For each treatment group, triplicate 25-g samples were weighed into sterile filter bags then the bags were sealed and stored at 10°C for 24 h.

   - After 24 h, 50 mL of sterile peptone water was added to each re-inoculated sample. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

8) The number of viable *Campylobacter* was determined by plating 0.1 mL aliquots of the stomached meat/peptone water mixture and a 1:10 dilution (in PBS) onto separate Campy CVA agar plates. The plates were incubated (42°C, 10% CO_2, 5% O_2, 85% N_2) for 48 h, and the CFU/g of sample were calculated after counting the colonies, as follows:

$$\frac{CFU\ recovered}{g\ of\ treated\ poultry} = \frac{CFU}{0.1\ mL\ plated} \times \frac{50\ mL\ peptone\ water}{25\ g\ sample}\ (\div\ dilution)$$

11 RESULTS

11.1 RAW DATA

Table 1 Raw Data for Study #CS20G22MC

Challenge with *Campylobacter*	Bacterial challenge	Treatment	Treatment time (h)	Bacterial re-challenge	Number of replicates	CFU/g recovered
1st Challenge	Yes	Control	6	No	3	6360, 6800, 5720
	Yes	~7x10^7 PFU/g CampyShield™	6	No	3	1480, 840, 1680
	Yes	Control	24	No	3	1780, 2420, 1940
	Yes	~7x10^7 PFU/g CampyShield™	24	No	3	420, 360, 520
	Yes	Control	48	No	3	1280, 1040, 1240
	Yes	~7x10^7 PFU/g CampyShield™	48	No	3	320, 240, 440
	No	Untreated Control	NA	No	1	0
2nd Challenge	Yes	Control	48	Yes	3	2660, 2260, 2640
	Yes	~7x10^7 PFU/g CampyShield™	48	Yes	3	2260, 2500, 2320

11.2 TABULAR PRESENTATION OF RESULTS

Table 2 Tabular Summary of the Results for Study #CS20G22MC

Challenge with *Campylobacter*	Treatment	Treatment time (h)	Number of replicates	Mean CFU/g	Average Percent reduction	Average Log reduction	Significance at p<0.05
1st Challenge	Control	6	3	6293	NA	NA	
	~7x10^7 PFU/g CampyShield™	6	3	1333	79%	0.67	Yes (p<0.001)
	Control	24	3	2047	NA	NA	
	~7x10^7 PFU/g CampyShield™	24	3	433	79%	0.67	Yes (p<0.001)
	Control	48	3	1187	NA	NA	
	~7x10^7 PFU/g CampyShield™	48	3	333	72%	0.55	Yes (p<0.001)
2nd Challenge	Control	48	3	2520	NA	NA	
	~7x10^7 PFU/g CampyShield™	48	3	2360	6%	0.03	No (p=0.49)

11.3 GRAPHICAL PRESENTATION OF RESULTS

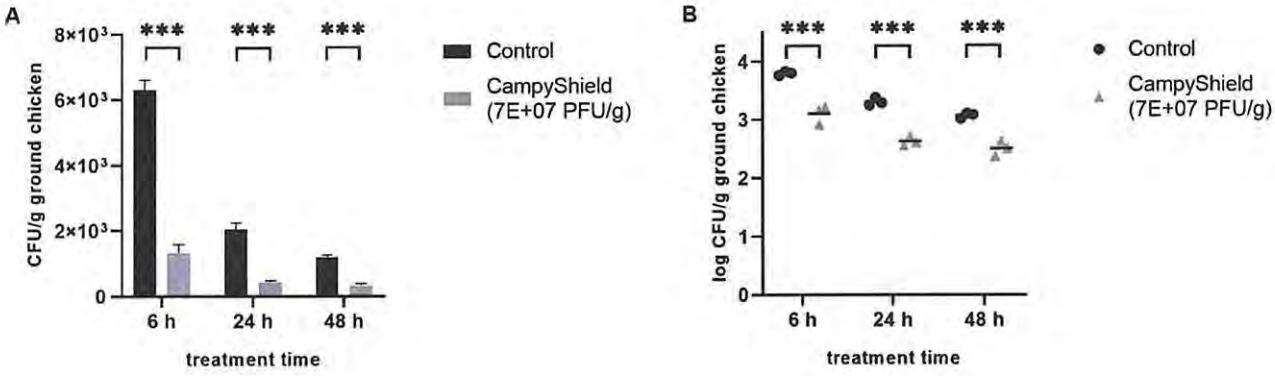

Figure 1 Overall reduction in *Campylobacter* levels on ground chicken by CampyShield™ (~7x10^7 PFU/g) compared to control after 6 h, 24 h, and 48 h. A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. (*** = p<0.001).

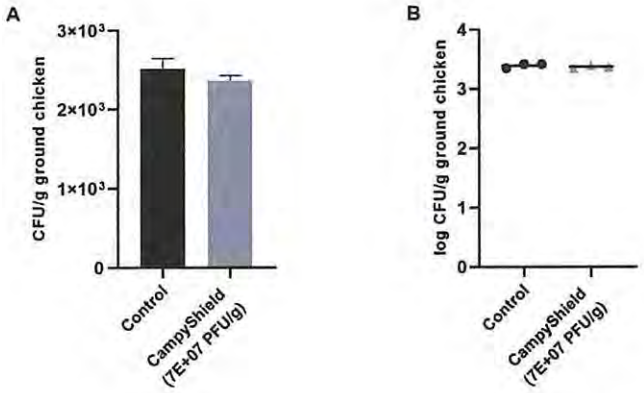

Figure 2 Populations of *Campylobacter* recovered 24 h after recontamination of ground chicken treated previously with control or CampyShield™ CampyShield™ compared to control after recontamination (1x10^4 CFU/g) post-treatment A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. No significant difference in populations recovered from the two treatments was observed.

11.4 STATISTICAL ANALYSIS

Reduction of Campylobacter by CampyShield™: The efficacy of the CampyShield™ treatment in reducing the number of viable *Campylobacter* cells in the experimentally contaminated ground chicken was evaluated by comparing the data obtained from control samples with the CampyShield-treated samples.

Statistical analysis was performed using GraphPad Prism for Windows (version 8.0.2; GraphPad Software; San Diego, CA; www.graphpad.com). The data was analyzed using Two-Way Analysis of Variance (Two-Way ANOVA). Sidak's Multiple Comparison test was performed to compare the treatment effect at 6 h, 24 h, and 48 h treatment time. A P value <0.05 was considered statistically significant.

ANOVA

H_0: The mean numbers of *Campylobacter* recovered from Control and CampyShield™ treatments are the same.

H_1: The mean numbers of *Campylobacter* recovered from Control and CampyShield™ treatments are different.

P value

The P value is <0.001, considered highly significant (H_0 rejected; H_1 accepted).

In summary, the mean populations of *Campylobacter* recovered from ground chicken are significantly different between the Control and CampyShield™ treatments.

The effect of CampyShield™ over treatment duration (i.e., 6 h vs. 24 h vs. 48 h) was determined using a two-way ANOVA to analyze the interaction between the two variables (treatment and time).

ANOVA

H_0: CampyShield™ has the same effect on *Campylobacter* populations at all timepoints.

H_1: CampyShield™ does not have the same effect on *Campylobacter* populations at all timepoints.

P value

The P value is 0.50, considered not significant (H_0 accepted).

In summary, increasing treatment time does not significantly increase the efficacy of CampyShield™ at reducing *Campylobacter* on ground chicken.

Reduction of Campylobacter following recontamination by CampyShield™: The continued technical effect of CampyShield™ after 48 h was evaluated by comparing the population of *Campylobacter* recovered after re-inoculation of Control and CampyShield™-treated samples. The statistical test used was an unpaired t-test.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

RECEIVED
AUG 18 2020
OFFICE OF FOOD ADDITIVE SAFETY

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*. OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Alexander Sulakvelidze	Vice President/Chief Scientific Officer

Organization *(if applicable)*: Intralytix, Inc.

Mailing Address *(number and street)*: 8681 Robert Fulton Dr

City	State or Province	Zip Code/Postal Code	Country
Columbia	Maryland	21046	United States of America

Telephone Number	Fax Number	E-Mail Address
410-625-2533	410-625-2506	asulakvelidze@intralytix.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title

Organization *(if applicable)*:

Mailing Address *(number and street)*:

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

CampyShield™ - Bacteriophage Preparation for reducing Campylobacter jejuni on foods

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway ☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media _____

3. For paper submissions only:

Number of volumes: _____

Total number of pages: _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

CampyShield™ is intended for use as an antimicrobial processing aid to control Campylobacter spp. on food when applied to food at up to 8 logs PFU/gram of food, including the following food categories:

- Raw and ground poultry
- Raw red meat products

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☒ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Intralytix
(name of notifier)

has concluded that the intended use*(s)* of CampyShield™ - Bacteriophage Preparation for reducing Campylobacter jejuni on foods
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Intralytix *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

8681 Robert Fulton Dr, Columbia, MD 21046
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Alexander Sulakvelidze Digitally signed by Alexander Sulakvelidze Date: 2020.08.14 17:27:34 -04'00'	Dr. Alexander Sulakvelidze, Vice President and CSO	08/14/2020

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	Intralytix_GRASNotice_Campyshield_Appendix_2020-08-14.pdf	GRAS Notice
	Intralytix_GRASNotice_CampyShield_2020-08-14.pdf	GRAS Notice
	Intralytix_GRASNotice_CampyShield_Cover-lett_2020-08-14.pdf	Administrative

From: Alexander Sulakvelidze
To: Hice, Stephanie
Subject: RE: GRN 000966 - Questions for Notifier
Date: Thursday, February 4, 2021 3:12:04 PM
Attachments: image001.png
Response to the FDA w Supporting Materials _ Feb 4 2021.pdf

Dear Stephanie,

Please see attached our responses to the FDA questions of January 26, 2021. As you will see in our responses, we will be doing some additional testing (e.g., for lead) and will provide the results to the FDA when they are available.

In the meantime, please let me know if you have any additional questions or if any of our answers requires further clarification.

Thank you!

Sandro Sulakvelidze

Alexander Sulakvelidze, Ph.D.
President and CEO
Intralytix, Inc.
8681 Robert Fulton Dr.
Columbia, MD 21046

Phone: 410-625-2533
Fax: 410-625-2506
E-mail: asulakvelidze@intralytix.com
www.intralytix.com

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Tuesday, January 26, 2021 8:07 AM
To: Alexander Sulakvelidze <asulakvelidze@intralytix.com>
Subject: GRN 000966 - Questions for Notifier

Dear Dr. Sulakvelidze,

During our review of GRAS Notice No. 000966, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Response to the FDA questions of January 26, 2021

Please see our responses to the questions below. The FDA questions are highlighted in **bold** font, followed by our responses.

1. Please clarify if the phage preparation is specific for *Campylobacter jejuni* only, or for *Campylobacter* spp. in general.

We anticipate that CampyShield™ will be effective against *Campylobacter* species in general, including *C. jejuni*.

2. Please confirm that the phage preparation is intended for use as an antimicrobial agent on raw and ground poultry and raw red meat products only.

Confirmed. The current GRAS notification is for use of CampyShield™ as an antimicrobial on raw and ground poultry and raw red meat products only.

3. Please state whether the three phages (J350, J375, and J386) have been deposited in a recognized culture collection and provide the deposit designation.

Yes, the three phages were deposited with the American Type Culture Collection (ATCC) on 9/22/2020, with the following identifiers:

J350 – CJLB-5
J375 – CJLB-10
J386 – CJLB-14

However, at the time of this writing (February 4, 2021), we have not yet received the patent depository numbers from the ATCC.

4. Please discuss whether the full genomic sequences of the three phages (J350, J375, and J386) are publicly available.

The full genomic sequences of the three phages (J350, J375, and J386) were deposited in GenBank on 10/2/2020. They have been issued the following Accession Numbers:

Phage Name	GenBank Accession number
CJLB-5 (J350)	MW057932
CJLB-10 (J375)	MW074124

CJLB-14 (J386) MW074126

The public release date for these sequences is January 2, 2023.

5. Please state whether the host *C. jejuni* strain has been deposited in a recognized culture collection and provide the deposit designation.

We have not deposited the host *C. jejuni* strains in a recognized culture collection. However, the CampyShield™ bacteriophages can be grown in several other strains previously deposited with the ATCC (e.g., ATCC 33292), that can be used by investigators worldwide to grow these phages for research purposes.

6. For the administrative record, please briefly specify how the purity of the host culture is ensured, and state whether the fermentation process is conducted in a contained, sterile environment.

Protocols are in place to maintain purity of the host culture. The master and production stocks of host strains are maintained in -80°C freezers. When used for production, host strains are handled in a Class II biosafety cabinet. Prior to each monophage production, the host strain is streaked out from the production seed stock/cell bank vial, which is then is discarded (i.e., there is no repeated entry into the production stock vial to minimize the risk of contamination, and no repeated freezing-thawing). The culture plate is inspected for homogeneity (i.e., lack of contamination) prior to being used in the monophage production. The host strain is then inoculated into sterile media in a suitable bioprocess vessel. After the host culture is infected with the monophage, the growth of the bacteria and subsequent lysis of that bacteria by the phage is monitored via spectrophotometer as an in-process control. Extensive QC protocols are in place to ensure the purity of both (i) the resulting monophage lots, and (ii) the CampyShield™ cocktail preparation after blending of monophages.

7. Please state whether any of the raw materials used in the fermentation are major allergens or derived from major allergens. If any of the raw materials used are major allergens or derived from major allergens, please discuss why these materials do not pose a safety concern.

Intralytix believes that CampyShield™ is free from major allergens. The vegan media we use for host growth/phage propagation does contain some hydrolyzed soy-based and wheat-based peptones. The media is manufactured (by Sigma-Aldrich) using fermentation-based hydrolysis

which significantly reduces the allergenicity of the soy and wheat proteins[1]. Furthermore, as this media is then used by Intralytix in another fermentation process to grow the host cells for phage propagation, the media components, including the soy- and wheat-based peptones, are further utilized by the host bacterium and degraded during this process. Following the fermentation step, we employ extensive filtration/concentration and washing steps (using 100 kDa filters) to purify component monophages. Thus, in the event any of the hydrolyzed soy- or wheat-based peptones are remaining after fermentation, they are expected to be removed during these steps. Based on the published literature [2], greater than 95% of the enzymatic hydrolysate from soy and wheat proteins present in the media are <15kDa in size; thus, they will be removed during the filtration and washing steps, as they pass through the 100 kDa filters. Taken together, these three processes/factors support the idea that CampyShield™ is free from all known major allergens (i) Sigma-Aldrich using hydrolyzed ingredients for media preparation (which significantly reduces the allergenicity of the soy and wheat proteins[3]), (ii) further hydrolyzation by the host cell culture (media components, including the soy- and wheat-based peptones, are further consumed and degraded by the host culture), and (iii) extensive filtration and washing of CampyShield™ monophages with 100 kDa filters that remove any remaining allergens present that are smaller than 100 kDa. Nevertheless, we will test the three non-consecutive lots of CampyShield we plan to generate (see our response to Question # 10 below) for the presence of known allergens and will provide testing results to the FDA when they are available.

8. Please include a specification for lead in Table 2 (page 5).

Intralytix has conducted a risk assessment (including for the presence of lead) regarding the components of the product and the process controls in place when manufacturing the product. We have concluded the risk for introduction of lead into the product or process is extremely low, if not non-existing. The major component of the manufacturing process for the product is in-house generated RO/DI water that is maintained by a 3rd party on a quarterly schedule and designed to remove impurities including lead, as shown in the included Neu-Ion deionization process worksheet. The absence of lead is confirmed through Intralytix's water monitoring program which includes periodic testing of the water for specific heavy metals including lead. Moreover, our risk assessment is further supported by the direct chemical analysis of numerous phage production lots

[1] *Meinlschmidt et. al. Food Science & Nutrition* ***2016,*** *4 (1), 11-23; Wilson et. al. Nutrition Reviews 2005, 63 (2), 47-58; Li et. al. Food Chemistry 2016, 196, 1338-1345; Leszczyńska et. al. Food and Agricultural Immunology 2009, 20 (2), 139-145*)

[2] *Netto, F. M.; Galeazzi, M. A. M., Production and Characterization of Enzymatic Hydrolysate from Soy Protein Isolate. LWT - Food Science and Technology* ***1998,*** *31 (7), 624-631; Lee, J.-Y.; Lee, H. D.; Lee, C.-H., Characterization of hydrolysates produced by mild-acid treatment and enzymatic hydrolysis of defatted soybean flour. Food Research International* ***2001,*** *34 (2), 217-222; Wang, J.-s.; Zhao, M.-m.; Zhao, Q.-z.; Bao, Y.; Jiang, Y.-m., Characterization of Hydrolysates Derived from Enzymatic Hydrolysis of Wheat Gluten. Journal of Food Science* ***2007,*** *72 (2), C103-C107.*

[3] *Meinlschmidt et. al. Food Science & Nutrition* ***2016,*** *4 (1), 11-23; Wilson et. al. Nutrition Reviews 2005, 63 (2), 47-58; Li et. al. Food Chemistry 2016, 196, 1338-1345; Leszczyńska et. al. Food and Agricultural Immunology 2009, 20 (2), 139-145*)

(CampyShield, and all commercial phage products currently marketed by Intralytix for food safety applications), all of which show absence of lead in our phage products. Thus, we do not believe that including specification for lead for CampyShield is warranted. We do commit to (i) testing the three non-consecutive lots of CampyShield we plan to generate (see our response to Question # 10 below) for the presence of lead and will provide testing results to the FDA when they are available, and (2) continued regular testing of our water (as the potential source of lead) and will notify the FDA should lead be detected at any time in the future. In the meantime, we have included the Howard County yearly water report, which shows the water supplied to our building is below detection limits, as well as our most recent RO/DI water system report.

9. Please provide complete and appropriate citations for the analytical methods used to analyze for the specification parameters and indicate that the methods are validated for their intended purpose. If an internally-developed method is used, please indicate that it has been validated for the intended purpose.

- Phage Potency (PFU/mL) is determined using a standard plaque assay. The method is well described in several book chapters and peer reviewed publications. Some examples are listed below.

1. Magnone, J. P.; Marek, P. J.; Sulakvelidze, A.; Senecal, A. G., Additive approach for inactivation of Escherichia coli O157:H7, Salmonella, and Shigella spp. on contaminated fresh fruits and vegetables using bacteriophage cocktail and produce wash. *J Food Prot* **2013,** *76* (8), 1336-41.
2. Anderson, B.; Rashid, M. H.; Carter, C.; Pasternack, G.; Rajanna, C.; Revazishvili, T.; Dean, T.; Senecal, A.; Sulakvelidze, A., Enumeration of bacteriophage particles. Bacteriophage 2011, 1 (2), 86-93.
3. Ács, N.; Gambino, M.; Brøndsted, L., Bacteriophage Enumeration and Detection Methods. *Frontiers in Microbiology* **2020,** *11* (2662).
4. Adams, M. H. (1959). Enumeration of bacteriophage particles. Bacteriophages. London, Interscience Publishers, Ltd.**:** 27-34.

- Microbial Purity is determined using an internally developed and verified method in accordance with 21 CFR §610.12.

- Endotoxin testing is conducted using FDA approved Endosafe PTS™, utilizing chromogenic Limulus amebocyte lysate (LAL) portable endotoxin detection system.

- Identity test is an in-process control. During manufacture, each component monophage is verified to be correct by two employees, who check both the lot number and volume added. This method is supported by the direct titration of the product (Potency Test) which confirms the correct total number of phages were added during mixing.

- Chemical analysis for lead is conducted using EPA method ICPMS (reference EPA 200.2/EPA 200.8, v. 5.4 1994).

10. Please provide results from a minimum of three (preferably five) non-consecutive batches to demonstrate that the phage preparation can be manufactured to meet the provided specifications listed in Table 2 (page 5). The notifier does not need to provide the results of batch analyses for lead if the batches in Table 3 (page 5) are non-consecutive and meet the specification limit set for lead.

Specification for CampyShield included in Table 2 were obtained by analyzing three *consecutive* lots of CampyShield. Therefore, in response to the FDA request, we will prepare three *non-consecutive* batches of CampyShield and will provide the information, as shown in the following table, when it is available.

Table 1 Product Batch specifications for CampyShield

Parameter	Batch Results			CampyShield™ Specification
	Lot #A	Lot #C	Lot #E	
Potency (PFU/mL)				≥10.0 log10 PFU/mL
Microbial purity				No growth
Endotoxin Content (EU/mL)				≤ 25,000 EU/mL (at ca. 9.0 log10 PFU/mL)
Identity				All phages included

In addition, in order to support our responses to questions 7 and 8, we will perform allergen testing and lead testing, respectively, on these lots as well.

11. For the administrative record, please provide the date (month and year) the literature search was performed.

The literature search was performed in July 2020.

12. Please provide a discussion of the scientific literature regarding the use of phage to control for the presence of *Campylobacter* spp.

Please see below a summary of scientific literature regarding the use of phages to reduce *Campylobacter* contamination of foods.

- Atterbury *et al*[4] tested the ability of *Campylobacter* phages to reduce the not actively replicating population of *C. jejuni* on chicken skin surface. Application of 10^7 PFU per 2 cm^2 area resulted in 1.1-1.3 log reduction in *Campylobacter* population at 4°C. The effect of phages was even more pronounced at -20°C, resulting in 2.3-2.5 log CFU reduction for different inoculum levels.
- In another study[5] , application of 10^6 PFU/cm^2 *Campylobacter* specific lytic phage resulted in ca. 95% reduction in *Campylobacter* counts on chicken skin.
- In yet another study, the authors reported isolation of 26 *Campylobacter* phages of the *Myoviridae* family. Nineteen of the 26 phages were examined for their ability to reduce *C. jejuni* in an *in vitro* assay. Each of these 19 phages reduced the viable *C. jejuni* counts by 1-3 log after 6-12 h at 42°C. The authors concluded that the phages can be used as biocontrol agents to reduce *C. jejuni* contamination[6] .
- One study isolated *C. jejuni* and lytic bacteriophages from chicken livers[7]. Most of the isolated bacteriophages were found to have a narrow host range. Application of one of the bacteriophages with a broader host range resulted in 0.7 log reduction in chicken liver suspensions.
- In experiments with raw and cooked beef samples[8], application of *Campylobacter* bacteriophages at ca. 10,000 multiplicity of infection (MOI) reduced the ca. 10^4 CFU/cm^2 *C. jejuni* population by 1.5 log and 2.2 log at 5 °C and 24 °C in 24h, respectively. The bacteriophages were also effective even when low counts (≤100 CFU/cm^2) of *C. jejuni* were present on meat samples; the reductions observed were 0.6 log when bacteriophages were applied at 10,000 MOI for 2h. All the reductions were statistically significant

[4] Atterbury, R. J.; Connerton, P. L.; Dodd, C. E. R.; Rees, C. E. D.; Connerton, I. F., Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **2003,** *69* (10), 6302-6306

[5] Goode, D.; Allen, V. M.; Barrow, P. A., Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Appl. Environ. Microbiol.* **2003,** *69* (8), 5032-5036

[6] Furuta, M.; Nasu, T.; Umeki, K.; Hoang Minh, D. U. C.; Honjoh, K.-I.; Miyamoto, T., Characterization and application of lytic bacteriophages against *Campylobacter jejuni* isolated from poultry in Japan. *Biocontrol Sci.* **2017,** *22* (4), 213-221.

[7] Firlieyanti, A. S.; Connerton, P. L.; Connerton, I. F., Campylobacters and their bacteriophages from chicken liver: The prospect for phage biocontrol. *Int. J. Food Microbiol.* **2016,** *237*, 121-127.

[8] Bigwood, T.; Hudson, J. A.; Billington, C.; Carey-Smith, G. V.; Heinemann, J. A., Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiol.* **2008,** *25* (2), 400-406.

demonstrating that bacteriophage application was effective in reducing *C. jejuni* contamination of raw and cooked red meat.

13. In Section 6.1.2 (page 20), the notifier lists the Code of Federal Regulations (CFR) citation for potassium chloride as 21 CFR 582.5622. We note that 21 CFR 582 corresponds to substances that are generally recognized as safe (GRAS) for use in animal drugs, feeds, and related products. As such, the appropriate CFR citation for potassium chloride is 21 CFR 184.1622, which corresponds to the listing of specific substances affirmed as GRAS for use in human conventional foods. For the administrative record, please make a statement that corrects this reference.

Thank you for pointing out this inadvertent discrepancy. The correct citation for potassium chloride is 21 CFT §184.1622.

14. Please provide the remaining portion of "Statistical Analysis" (Section 11.4), as well as "Brief Discussion of Results and Study's Conclusions" (Section 11.5), "Summary Conclusion of the Study" (Section 12) and the "Signatures" page (Section 13) (pages 9-10) for Study# CS20G22MC (Appendix 1.4), as they are missing from the notice.

The Complete Study #CS20G22MC is enclosed, with all the requested sections (including statistical analysis) included.

Appendix 1.4:
Study
#CS20G22MC

Evaluation of the ability of CampyShield™ to (i) reduce *Campylobacter* contamination of ground chicken, and (ii) provide protection against recontamination of ground chicken with *Campylobacter*

Study # CS20G22MC

Intralytix
8681 Robert Fulton Dr.
Columbia, MD 21046
www.intralytix.com

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1 STUDY TITLE

Evaluation of the ability of CampyShield™ to (i) reduce *Campylobacter* contamination of ground chicken and (ii) provide protection against recontamination of ground chicken with *Campylobacter*

2 STUDY DIRECTOR

Amit Vikram, Ph.D.

3 STUDY PERSONNEL

Name:	Title:	Role:
Mary Theresa Callahan, MS	Research Scientist	Hands-on-research / Data Review / Report Assembly
Amit Vikram, Ph.D.	Senior Research Scientist	Study Director / Data Review /Report Assembly
Joelle Woolston, MS	Director, Laboratory Operations	Data review / Report Assembly

4 PERFORMING LABORATORY

Intralytix, Inc.
Research and Development
8681 Robert Fulton Dr
Columbia, MD 21046

5 STUDY OBJECTIVE

To determine whether application of CampyShield™ (i) reduces the number of viable *Campylobacter jejuni* in experimentally contaminated ground chicken when applied at a rate of 4 mL per lb of poultry, and (ii) provides a residual technical effect against recontamination of ground chicken with *Campylobacter.*

6 TEST MATRIX

Fresh ground chicken was purchased on the day of the experiment at a Columbia, MD area supermarket. The ground chicken was not pre-treated prior to our studies.

7 CAMPYSHIELD™ LOT AND APPLICATION RATE

- CampyShield™ Lot #0420F3010testA
- Titer: $7.6x10^9$ PFU/mL
- The application rate was ca. 4 mL CampyShield™ per pound of ground chicken.
- CampyShield™ was applied using Burkle 20 mL spray bottles with pump vaporizer (Burkle Inc, Bohemia, NY)

8 BACTERIAL STRAIN USED TO EXPERIMENTALLY CONTAMINATE GROUND CHICKEN

The ground chicken test matrix was experimentally contaminated with a single *Campylobacter* strain:

- *CJ160: Campylobacter jejuni* subsp. *jejuni* strain ATCC33292, which was isolated from human feces

Shortly before performing the study, the strain was thawed and grown (42 ± 2°C; 10% CO_2, 5% O_2, 85% N_2) in cation-adjusted brain heart infusion (cBHI) broth for 24 h, which corresponds to ca. $7x10^9$ CFU/mL. The culture was diluted 1000-fold in phosphate buffered saline (PBS) prior to application to ground chicken.

The ground chicken was experimentally contaminated by ca. $1x10^4$ CFU of *Campylobacter* / g of ground chicken.

9 MEDIA AND REAGENTS

- BHI broth (Sigma-Aldrich, Burlington, MA; catalog # 53286-500G)
- $CaCl_2$ (Sigma-Aldrich, Burlington, MA; catalog # C-3306)
- $MgSO_4$ (Fisher Chemicals, Hampton, NH; catalog # M65-500)
- Campy CVA agar (Becton, Dickinson and Co., Sparks, MD; catalog # 297246)
- Peptone water (Becton, Dickinson and Co., Sparks, MD; catalog # 212367)
- PBS (pH 7.4) (Life Technologies, Grand Island, NY; catalog # 10010031)

10 GENERAL OUTLINE OF STUDY

1) Two 325-g samples of ground chicken were weighed into sterile mixing bowls. An additional 25-g sample for each experiment was not treated with bacterial cultures as the uncontaminated, untreated control.

2) The challenge dose of bacteria was applied onto the ground chicken surface via pipette and the bacteria were allowed to colonize the ground chicken matrix at room temperature (approximately 22°C) for 20 min. Then, samples were mixed at the lowest mixing speed ("stir") for 10 min using a KitchenAid™ stand mixer with the flat beater attachment.

3) PBS (control) or CampyShield™ was applied to the samples as described in Section 7. Samples were sprayed with 2.86 mL of either PBS (control) or CampyShield™. The final concentration of CampyShield™ was ca. 6.7×10^7 PFU/g.

4) Following treatment, samples sat for 10 min at room temperature, then were mixed for an additional 10 min.

5) After mixing, 25 g samples (n=9) were weighed from each treatment group into separate sterile filter bags then the bags were sealed and placed at 10°C. The remaining 100 g of ground chicken was covered in plastic wrap and placed at 10°C.

6) At 6 h, 24 h, and 48 h post-treatment, triplicate samples bags from each treatment group were removed and 50 mL of sterile peptone water was added. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

7) At 48 h post-treatment, the 100 g samples from Step 5 (inoculated and treated ground chicken samples) were re-inoculated with 100 µL of a freshly prepared CJ160 inoculum, as described in Section 8. The resulting bacterial challenge was ca. 1×10^4 CFU/g of ground chicken.

    - Following inoculation, samples were incubated at room temperature for 20 min, and then were mixed for an additional 10 min on the lowest mixing setting. For each treatment group, triplicate 25-g samples were weighed into sterile filter bags then the bags were sealed and stored at 10°C for 24 h.

    - After 24 h, 50 mL of sterile peptone water was added to each re-inoculated sample. The bags were stomached at 200 rpm for one minute using a Stomacher 400 Circulator (Port Saint Lucie, FL).

8) The number of viable *Campylobacter* was determined by plating 0.1 mL aliquots of the stomached meat/peptone water mixture and a 1:10 dilution (in PBS) onto separate Campy CVA agar plates. The plates were incubated (42°C, 10% CO_2, 5% O_2, 85% N_2) for 48 h, and the CFU/g of sample were calculated after counting the colonies, as follows:

$$\frac{CFU\ recovered}{g\ of\ treated\ poultry} = \frac{CFU}{0.1\ mL\ plated} \times \frac{50\ mL\ peptone\ water}{25\ g\ sample}\ (\div dilution)$$

11 RESULTS

11.1 RAW DATA

Table 1 Raw Data for Study #CS20G22MC

Challenge with *Campylobacter*	Bacterial challenge	Treatment	Treatment time (h)	Bacterial re-challenge	Number of replicates	CFU/g recovered
1st Challenge	Yes	Control	6	No	3	6360, 6800, 5720
	Yes	~7x10^7 PFU/g CampyShield™	6	No	3	1480, 840, 1680
	Yes	Control	24	No	3	1780, 2420, 1940
	Yes	~7x10^7 PFU/g CampyShield™	24	No	3	420, 360, 520
	Yes	Control	48	No	3	1280, 1040, 1240
	Yes	~7x10^7 PFU/g CampyShield™	48	No	3	320, 240, 440
	No	Untreated Control	NA	No	1	0
2nd Challenge	Yes	Control	48	Yes	3	2660, 2260, 2640
	Yes	~7x10^7 PFU/g CampyShield™	48	Yes	3	2260, 2500, 2320

11.2 TABULAR PRESENTATION OF RESULTS

Table 2 Tabular Summary of the Results for Study #CS20G22MC

Challenge with *Campylobacter*	Treatment	Treatment time (h)	Number of replicates	Mean CFU/g	Average Percent reduction	Average Log reduction	Significance at p<0.05
1st Challenge	Control	6	3	6293	NA	NA	
	~7x10^7 PFU/g CampyShield™	6	3	1333	79%	0.67	Yes (p<0.001)
	Control	24	3	2047	NA	NA	
	~7x10^7 PFU/g CampyShield™	24	3	433	79%	0.67	Yes (p<0.001)
	Control	48	3	1187	NA	NA	
	~7x10^7 PFU/g CampyShield™	48	3	333	72%	0.55	Yes (p<0.001)
2nd Challenge	Control	48	3	2520	NA	NA	
	~7x10^7 PFU/g CampyShield™	48	3	2360	6%	0.03	No (p=0.49)

11.3 GRAPHICAL PRESENTATION OF RESULTS

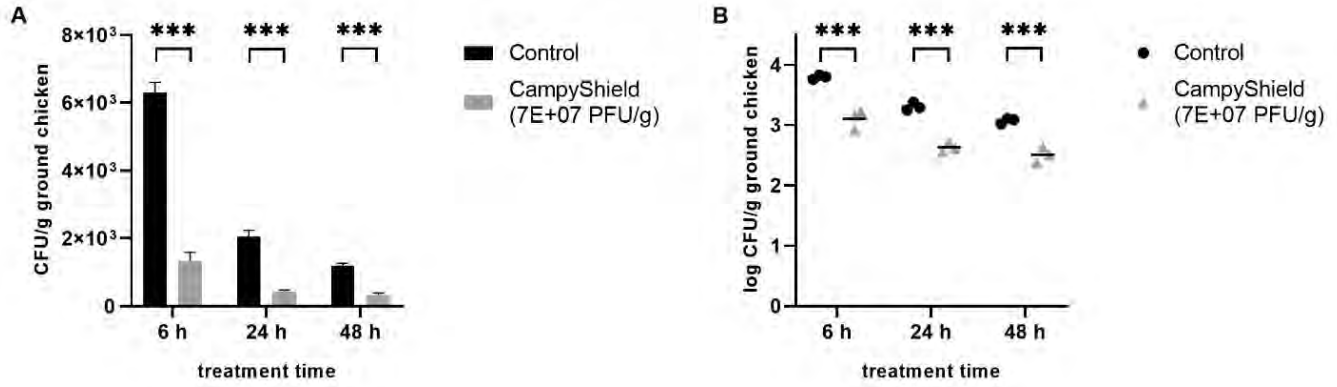


Figure 1 Overall reduction in *Campylobacter* levels on ground chicken by CampyShield™ (~7x10^7 PFU/g) compared to control after 6 h, 24 h, and 48 h.
A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. (* = p<0.001).**

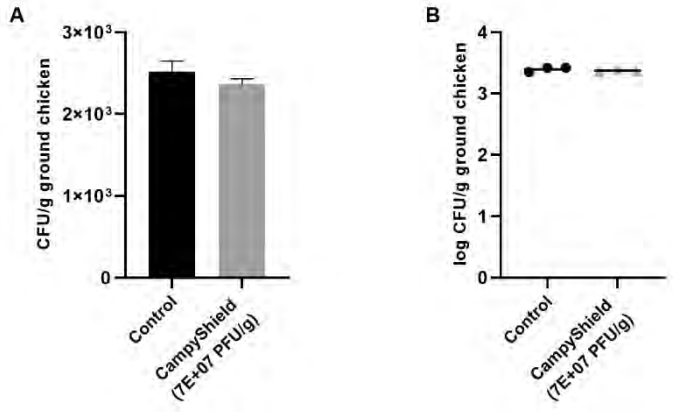


Figure 2 Populations of *Campylobacter* recovered 24 h after recontamination of ground chicken treated previously with control or CampyShield™
CampyShield™ compared to control after recontamination (1x10^4 CFU/g) post-treatment A) presented linearly scaled on y-axis, error bars indicate standard error of the mean. B) presented in log scale on y-axis, bars indicate the mean. No significant difference in populations recovered from the two treatments was observed.

11.4 STATISTICAL ANALYSIS

Reduction of Campylobacter by CampyShield™: The efficacy of the CampyShield™ treatment in reducing the number of viable *Campylobacter* cells in the experimentally contaminated ground chicken was evaluated by comparing the data obtained from control samples with the CampyShield-treated samples.

Statistical analysis was performed using GraphPad Prism for Windows (version 8.0.2; GraphPad Software; San Diego, CA; www.graphpad.com). The data was analyzed using Two-Way Analysis of Variance (Two-Way ANOVA). Sidak's Multiple Comparison test was performed to compare the treatment effect at 6 h, 24 h, and 48 h treatment time. A P value <0.05 was considered statistically significant.

ANOVA

H_0: The mean numbers of *Campylobacter* recovered from Control and CampyShield™ treatments are the same.

H_1: The mean numbers of *Campylobacter* recovered from Control and CampyShield™ treatments are different.

P value

The P value is <0.001, considered highly significant (H_0 rejected; H_1 accepted).

In summary, the mean populations of *Campylobacter* recovered from ground chicken are significantly different between the Control and CampyShield™ treatments.

The effect of CampyShield™ over treatment duration (i.e., 6 h vs. 24 h vs. 48 h) was determined using a two-way ANOVA to analyze the interaction between the two variables (treatment and time).

ANOVA

H_0: CampyShield™ has the same effect on *Campylobacter* populations at all timepoints.

H_1: CampyShield™ does not have the same effect on *Campylobacter* populations at all timepoints.

P value

The P value is 0.50, considered not significant (H_0 accepted).

In summary, increasing treatment time does not significantly increase the efficacy of CampyShield™ at reducing *Campylobacter* on ground chicken.

Reduction of Campylobacter following recontamination by CampyShield™: The continued technical effect of CampyShield™ after 48 h was evaluated by comparing the population of *Campylobacter* recovered after re-inoculation of Control and CampyShield™-treated samples. The statistical test used was an unpaired t-test.

ANOVA

H_0: The sample means of *Campylobacter* recovered from re-contaminated Control and CampyShield™ treatments are the same.

H_1: The sample means of *Campylobacter* recovered from re-contaminated Control and CampyShield™ treatments are different.

P value

The P value is 0.49, considered not significant (H_0 accepted).

In summary, no continued technical effect of CampyShield™ was observed after 48 h. The populations of *Campylobacter* recovered after recontamination of CampyShield™-treated samples was similar (p>0.05) to the populations recovered from re-contaminated control samples.

11.5 BRIEF DISCUSSION OF RESULTS AND STUDY'S CONCLUSIONS

- Applying $7x10^9$ PFU/mL CampyShield™ to ground chicken – at the rate of 4.0 mL per lb of ground chicken – significantly reduced the number of viable *Campylobacter* by ca. 79% after 6 h, 79% after 24 h, and 72% after 48 h of incubation at 10°C. The observed reductions were statistically significant (P<0.001).

- Increasing CampyShield™ treatment time from 6 h to 24 h or 48 h did not significantly affect the observed reduction of *Campylobacter* in ground chicken (P>0.05).

- CampyShield™ does not provide long-term protection against recontamination of ground chicken with *Campylobacter,* i.e. there is no continued technical effect of CampyShield™. There was no significant difference between the populations of *Campylobacter* recovered from Control and CampyShield™-treated samples following recontamination of ground chicken (P>0.05).

12 SUMMARY CONCLUSION OF THE STUDY

CampyShield™ significantly reduced the *Campylobacter* population in ground chicken samples by ca. 77% when it was applied to the experimentally contaminated meat.

Increasing the treatment time beyond 6 h did not increase the efficacy of CampyShield™ against *Campylobacter*.

These results agree with the results reported in CS20G20MCB, demonstrating that CampyShield™ significantly reduced *Campylobacter* contamination of ground chicken upon initial application.

The application of CampyShield™ at the start of experiment did not impact the *Campylobacter* population on ground chicken re-contaminated 48 h after initial treatment, indicating CampyShield does not demonstrate a continued technical effect against *Campylobacter* contamination after the initial phage application.

13 SIGNATURES

[redacted]

Mary Theresa Callahan, MS
Research Scientist
Hands on Research

14th August 2020
Date

[redacted]

Amit Vikram, PhD
Senior Research Scientist
Study Director

14th August 2020
Date

[redacted]

Joelle Woolston, MS
Director, Laboratory Operations

14-Aug-2020
Date

NEU·ION
HIGH PURITY WATER SYSTEMS

Intralytix Inc
8681 Robert Fulton Drive
Columbia MD 21046

R.O. - D.I. CHECKLIST

RAW INCOMING FEEDWATER ANALYSIS

Inlet Feed Quality	300us
Hardness	10gpg
Iron Level	Nd
pH Level of Feed	7.5
CL2 :Level of Feedwater	1.4ppm

PRETREATMENT

Softener Type/Size	12x52 neuion
Salt Used This Period	6
Hardness	Nd
Turbidity Filter Type/Size	Na
Carbon Filter Type/Size	12x52neuion
Total Chlorine Level	Nd

FILTRATION & HOUSINGS

Cartridge Filter Type/Size	17103/17505
Replace Filter Element	Yes
Post Filter Type/Size	Mpn0.2-10s3s(4)
Replace Filter Element	Na
Vent Filter Type/Size	Na
Vent Filter Last Changed	Na

REVERSE OSMOSIS UNIT

RO Type - Model	E44400dlx4606lefrp
Check High Level Control	Yes
Inlet Pressure	60psi
Pump Pressure	190psi
Membrane Pressure	190psi
Concentrate Flow Rate	.5gpm
Permeate Flow Rate	2.0gpm
Permeate Quality+% rejection	1.5us 99%

Account #: INTRA01

DELIVERY SYSTEM

Pump Type	Crne-9
Noise Level	Normal
Pump Pressure	Na
Post Valve Pressure	70psi
Loop Return Pressure	10psi

POLISHING SYSTEM

System Type	204mb47
Carbon Last Replaced	Na
Resin Last Replaced	18dec20
Final Quaity	Above 2megohms

ULTRAVIOLET UNIT

Model	lj151177
Lamp On	Yes
Replace Lamp On	Na
Replace Quartz Sleeve	Na
Hour Meter/Irradiation	Na

Comments:

Service Representative Signature

Customer Signature

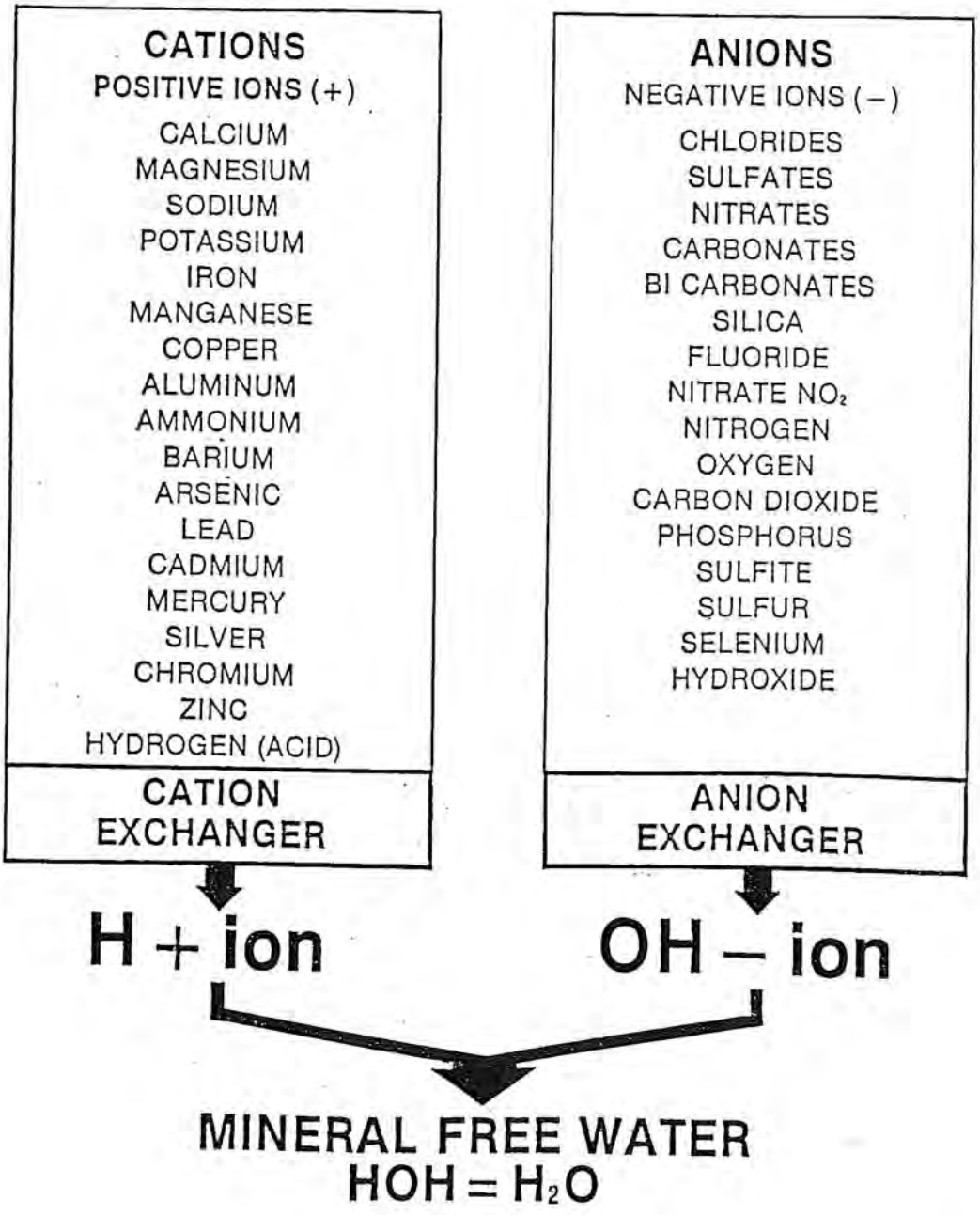
12/18/2020

Date

NEU-ION, INC
High Purity Water Systems

7200 Rutherford Road, Suite 100
Baltimore, MD 21244
(800) 678-4360 / (410) 944-5200
F: (410) 944-5289
Email: info@neu-ion.com

DEIONIZATION PROCESS



Howard County Department of Public Works

Annual Water Quality Report

Reporting Period January 1, 2019 to December 31, 2019

PSWID 0130002

Calvin Ball,
Howard County Executive

Howard County Drinking Water

Access to clean water is not only a human right but a critical foundation of our society. We don't take our access for granted and work hard every day to ensure high-quality drinking water for all our residents in Howard County. Our Bureau of Utilities is charged with conducting regular tests and publishing their results for the public.

This Consumer Confidence Report is a detailed summary of our community's drinking water quality. You can learn where your water is sourced, and how we ensure it is clean and safe.

We're deeply grateful to our Howard County employees who work to protect our water quality and provide uninterrupted service so that each time we turn on the tap – we know we're drinking clean water.

Howard County is pleased to present its 2020 Water Quality Report. This report is designed to inform residents about the quality and dependability of water and services provided to them every day. The goal of this report is to help readers better understand the efforts our water suppliers make to continually improve the water treatment process and protect our water resources. The county sources its water from the Liberty Reservoir on the North Branch of the Patapsco River and the Loch Raven Reservoir on the main stream of the Gunpowder Fall from Baltimore City and from the Patuxent River from the Washington Suburban Sanitary Commission. Here in Howard County, we work hard and are committed to ensuring the quality of your water.

DEAR VALUED CUSTOMER,

Our mission here at Howard County's Department of Public Works', Bureau of Utilities is to provide the highest quality, safest and most dependable drinking water to our customers – whether a county resident, business or visitor. In coordination with our regional water suppliers, the City of Baltimore and the Washington Suburban Sanitary Commission, we continuously strive to deliver the finest water supply service, even in times of crisis. In response to the COVID-19 pandemic, here in Howard County, we've taken the necessary steps to ensure we continue to provide reliable water service to our customers in the safest manner possible. This unprecedented time has required us to implement additional steps to protect our dedicated, essential staff members and the county's drinking water system. Our staff remains available 24/7, expertly assessing and maintaining the physical conditions of our water infrastructure, while overseeing our long-term capital improvement programming. We hope that through this report, you will gather a better understanding of our water services and the quality of the product we deliver to you daily. Should you have any questions, please do not hesitate to contact our Bureau of Utilities team at 410-313-4900 or visit us online at www.howardcountymd.gov/Departments/Public-Works/Bureau-Of-Utilities.

***Art Shapiro*, PE, PMP**

Chief, Bureau of Utilities

WHY WATER IS TESTED:

All sources of drinking water are subject to potential contamination by substances that are naturally occurring or manmade. These substances can be microbes, inorganic or organic chemicals and radioactive substances. As water travels over the land or underground, it can pick up substances or contaminants such as microbes, inorganic and organic chemicals, as well as radioactive substances, resulting from the presence of animals or from human activity. All drinking water, including bottled drinking water, may be reasonably expected to contain at least small amounts of some contaminants. The presence of contaminants does not necessarily indicate that the water poses a health risk.

Contaminants that could be present in source water include:

- Microbial contaminants, such as viruses and bacteria, which may come from sewage treatment plants, septic systems, agricultural livestock operations and wildlife.
- Inorganic contaminants, such as salts and metals, which can be naturally occurring or result from urban storm water runoff, industrial or domestic wastewater discharges, oil and gas production, mining, or farming.
- Pesticides and herbicides, which may come from a variety of sources such as agriculture, urban storm water runoff and residential uses.
- Organic chemical contaminants, including synthetic and volatile organic chemicals, which are by-products of industrial processes and petroleum production, and can also come from gas stations, urban storm water runoff and septic systems.

To ensure that tap water is safe to drink, the Environmental Protection Agency (EPA) sets regulations that limit the amount of certain contaminants in water provided by public water systems. Food and Drug Administration regulations set limits for contaminants in bottled water that must provide the same protection for public health.

The Maryland Department of the Environment (MDE) has completed a source water assessment of the water supplies that serve the City of Baltimore. The Source Water Assessment Program may be viewed at the MDE web site, http://www.mde.state.md.us/programs/Water/Water_Supply/ConsumerConfidenceReports/Documents/CCR2015/Howard/0130002_Howard_County.pdf.

More information about contaminants and potential health effects can be obtained by calling the EPA's Safe Drinking Water Hotline at 1-800-426-4791.

FOR MORE INFORMATION

If you have any questions about this report or concerning your water utility, please contact the County's Bureau of Utilities at 410-313-4900. We want our valued customers to be informed about their water utility. If you want to learn more, please attend any of our regularly scheduled Department of Public Works Board meetings. Please call 410-313-2330 for further information about these meetings.

Employees at the County's Bureau of Utilities work around the clock to provide top quality water to every tap. We ask that all our customers help us protect our water sources, which are the heart of our community, our way of life and our children's future.

TEST RESULTS – HOWARD COUNTY - PSWID 0130002

	Violation Y/N	Total Sample Collected	Total Coliform* Positive	E-coli** Positive	E-coli MCLG	E-coli MCLG
Microbiological Contaminants						
Routine Samples	N	1802	4	0	0	0
Repeat Sample	N	12	0	0	0	0

*Coliform bacteria—naturally present in the environment
** E-coli—pathogen from human and animal fecal waste

TEST RESULTS – OUR SUPPLIERS

	Baltimore City Supply				Washington Suburban Sanitary Commission Supply				
	Ashburton Plant		Montebello Plant						
Contaminant - Units	Violation Y/N	Level Detected	Viola-tion Y/N	Level Detected	Violation Y/N	Level Detected	MCLG	MCL	Likely Source of Contamination
Microbiological Contaminants									
Turbidity - NTU	N	0.06	N	0.21	N	0.03	1.00	TT= Filtration	Soil runoff
Radioactive Contaminants									
Beta/photon emitters pCi/l	Combined Radium 226/228		N	<4	N	4.6	0	50	Decay of natural and man-made deposits
Alpha/photon emitters pCi/l	N	1.6	N	<4	N	ND	0	50	Erosion of natural deposits
Inorganic Contaminants									
Antimony - ppb	N	<5	N	<5	N	ND	6	6	Discharge from petroleum refineries; fire retardants; ceramics; electronics; solder
Arsenic – ppb	N	<2	N	<2	N	ND	0	10	Erosion of natural deposits; runoff from orchards; runoff from glass And electronics production wastes
Barium – ppm	N	0.02	N	0.036	N	0.03	2	2	Discharge of drilling wastes; discharge from metal refineries; erosion of natural deposits
Beryllium – ppb	N	<0.5	N	<0.5	N	ND	4	4	Discharge from metal refineries And coal-burning factories; discharge from electrical, aerospace, And defense industries
Cadmium	N	<0.5	N	<0.5	N	ND	5	5	Erosion of natural deposits; runoff from orchards, runoff from glass & electronics production wastes
Chromium – ppb	N	<2	N	<2	N	ND	100	100	Discharge from steel and pulp mills; erosion of natural deposits
Copper – ppm	N	<.002	N	<.002	N	ND	1.3	AL=1.3	Corrosion of household plumbing systems; erosion of natural deposits; leaching from wood preservatives
Fluoride – ppm	N	1.03	N	1.07	N	0.7	4	4	Erosion of natural deposits; water additive which promotes strong teeth; discharge from fertilizer and aluminum factories
Lead – ppb	N	<2	N	<2	N	ND	0	AL=15	Corrosion of household plumbing systems, erosion of natural deposits
Mercury (inorganic) Ppb	N	<0.5	N	<0.5	N	ND	2	2	Erosion of natural deposits; discharge from refineries and factories; runoff from landfills; runoff from cropland
Nitrate (as Nitrogen) Ppm	N	2.87	N	1.97	N	1.5	10	10	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits
Nitrite (as Nitrogen) Ppm	N	<0.01	N	<0.01	N	ND	1	1	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits
Selenium – ppb	N	<5	N	<5	N	ND	50	50	Discharge from petroleum and metal refineries; erosion of natural deposits; discharge from mines
Thallium – ppb	N	<1	N	<1	N	ND	0.5	2	Leaching from ore-processing sites; discharge from electronics, glass, and drug factories
Synthetic Organic Contaminants including Pesticides and Herbicides									
2,4-D – ppb	N	<1.0	N	<1.0	N	ND	70	70	Runoff from herbicide used on row crops
2,4,5-TP (Silvex) - ppb	N	<1.0	N	<1.0	N	ND	50	50	Residue of banned herbicide
Alachlor – ppb	N	<2	N	<2	N	ND	0	2	Runoff from herbicide used on row crops
Atrazine – ppb	N	<2	N	<2	N	ND	3	3	Runoff from herbicide used on row crops
Benzo(a)pyrene – ppb	N	<0.2	N	<0.2	N	ND	0	0.2	Leaching from linings of water storage tanks and distribution lines
Carbofuran - ppb	N	<1.0	N	<1.0	N	ND	40	40	Leaching of soil fumigant used on rice and alfalfa
Chlordane - ppb	N	<2	N	<2	N	ND	0	2	Residue of banned termiticide
Dalapon – ppb	N	<4.0	N	<4.0	N	ND	200	200	Runoff from herbicide used on rights of way

KEY TABLE
In this table, you will find many terms and abbreviations you might not be familiar with. To help you better understand these terms, we've provided the following definitions:

Non-Detects (ND) - laboratory analysis indicates that the constituent is not detectable by the analytical instrument used

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Picocuries per liter (pCi/L) - picocuries per liter is a measure of the radioactivity in water.

Millirems per year (mrem/yr) - measure of radiation absorbed by the body.

Million Fibers per Liter (MFL) - million fibers per liter is a measure of the presence of asbestos fibers that are longer than 10 micrometers.

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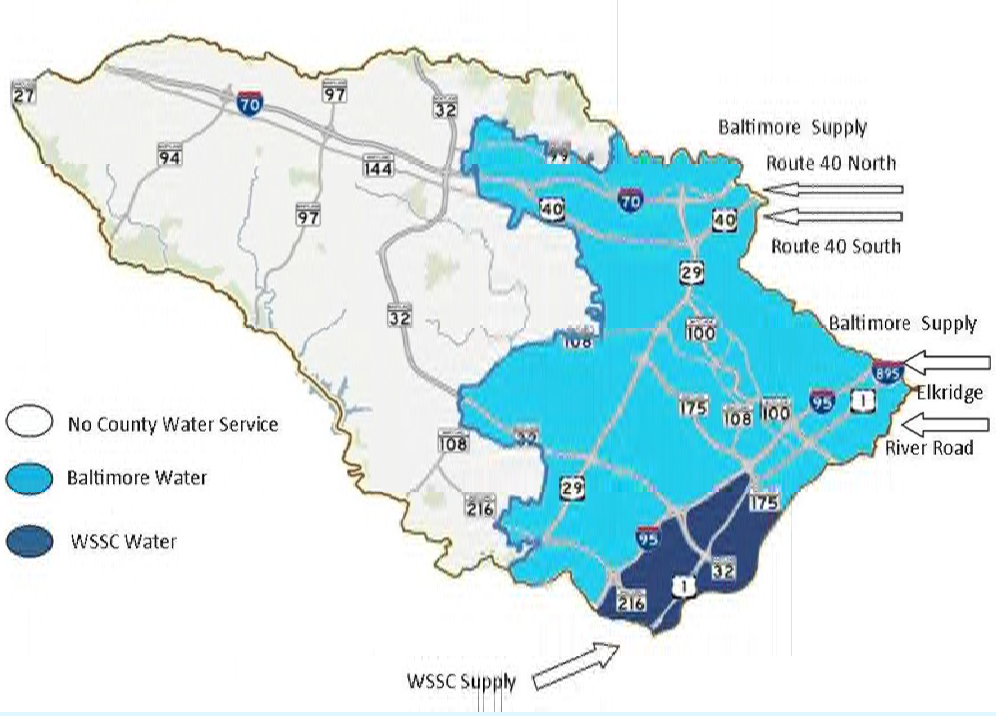
Dibromochloropropane -ppb	N	<0.02	N	<0.02	N	ND	0	0.2	Runoff/leaching from soil fumigant used on soybeans, cotton, pineapples, and orchards
Dinoseb – ppb	N	<1.0	N	<1.0	N	ND	7	7	Runoff from herbicide used on soybeans and vegetables
Endrin – ppb	N	<0.5	N	<0.5	N	ND	2	2	Residue of banned insecticide
Ethylene dibromide - ppb	N	<0.05	N	<0.05	N	ND	0	0.05	Discharge from petroleum refineries
Heptachlor - ppb	N	<0.4	N	<0.4	N	ND	0	0.4	Residue of banned termiticide
Heptachlor epoxide - ppb	N	<0.2	N	<0.2	N	ND	0	0.2	Breakdown of heptachlor
Hexachlorobenzene - ppb	N	<1.0	N	<1.0	N	ND	0	1	Discharge from metal refineries and agricultural chemical factories
Hexachlorocyclo-pentadiene - ppb	N	<0.5	N	<0.5	N	ND	50	50	Discharge from chemical factories
Lindane-ppb	N	<0.2	N	<0.2	N	ND	0.2	0.2	Runoff/leaching from insecticide used on cattle, lumber, gardens
Methoxychlor - ppb	N	<0.5	N	<0.5	N	ND	40	40	Runoff/leaching from insecticide used on fruits, vegetables, alfalfa, livestock
Oxamyl [Vydate]-ppb	N	<1.0	N	<1.0	N	ND	200	200	Runoff from Landfills; discharge of waste chemicals
Pentachlorophenol - ppb	N	<1.0	N	<1.0	N	ND	0	1	Discharge from wood preserving factories
Picloram – ppb	N	<2.0	N	<2.0	N	ND	500	500	Herbicide runoff
Simazine – ppb	N	<0.5	N	1.4	N	ND	4	4	Herbicide runoff
Volatile Organic Contaminants									
Benzene – ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from factories; leaching from gas storage tanks and Landfills
Carbon tetrachloride - ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from chemical plants And other industrial activities
Chlorobenzene – ppb	N	<0.5	N	<0.5	N	ND	100	100	Discharge from chemical and agricultural chemical factories
o-Dichlorobenzene - ppb	N	<0.5	N	<0.5	N	ND	600	600	Discharge from industrial chemical factories
p-Dichlorobenzene - ppb	N	<0.5	N	<0.5	N	ND	75	75	Discharge from industrial chemical factories
1,2 – Dichloroethane - ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from industrial chemical factories
1,1 – Dichloroethane - ppb	N	<0.5	N	<0.5	N	ND	7	7	Discharge from industrial chemical factories
cis-1,2-Dichloroethene - ppb	N	<0.5	N	<0.5	N	ND	70	70	Discharge from industrial chemical Factories
trans-1,2 Dichloroethene - ppb	N	<0.5	N	<0.5	N	ND	100	100	Discharge from industrial chemical factories
Dichloromethane– ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from pharmaceutical and chemical factories
1,2-Dichloropropane Ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from industrial chemical factories
Ethylbenzene – ppb	N	<0.5	N	<0.5	N	ND	700	700	Discharge from petroleum refineries
Haloacetic Acids, Total– ppb	N	55.0	N	55.0	N	41.0	0	60	By-product of drinking water chlorination
Styrene – ppb	N	<0.5	N	<0.5	N	ND	100	100	Discharge from rubber and plastic factories; leaching from landfills
Tetrachloroethylene – ppb	N	<0.5	N	<0.5	N	ND	0	5	Leaching from PVC pipes; discharge from factories and dry cleaners
1,2,4-Trichlorobenzene - Ppb	N	<0.5	N	<0.5	N	ND	70	70	Discharge from textile-finishing factories
1,1,1 – Trichloroethane - Ppb	N	<0.5	N	<0.5	N	ND	200	200	Discharge from metal degreasing sites and other factories
1,1,2 –Trichloroethane - Ppb	N	<0.5	N	<0.5	N	ND	3	5	Discharge from industrial chemical factories
Trichloroethene – ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from metal degreasing sites and other factories
TTHM - ppb [Total trihalomethanes]	N	77.0	N	77.0	N	62.0	0	80	By-product of drinking water chlorination
Vinyl Chloride - ppb	N	<0.5	N	<0.5	N	ND	0	2	Leaching from PVC piping; discharge from plastics factories
Toluene – ppb	N	<0.5	N	<0.5	N	ND	1000	1000	Discharge from petroleum factories
Xylenes – ppb	N	<0.5	N	<0.5	N	ND	10000	10000	Discharge from petroleum factories; discharge from chemical factories

TEST RESULTS - HOWARD COUNTY—PSWID 0130002						
Volatile Organic Chemicals						
Substance	MCLG	MCL	Range (LRAA)	Average	Violation	Major Sources
Total THM's	**60**	**80ppb**	20 - 59.1 ppb	41 ppb	No	Byproduct of drinking water chlorination
HAA(5)	**46**	**60ppb**	6.4 - 60.3 ppb	32 ppb	No	Byproduct of drinking water chlorination



WHERE YOUR WATER COMES FROM

If you live in the North Laurel area, east of I-95 and south of Patuxent Range Road, your water originates from the Washington Suburban Sanitary Commission in Laurel. If you live anywhere else in Howard County and are connected to the public water supply, your water originates from Baltimore City. As a "Consecutive Water System,"
Howard County purchases its water from Baltimore City and the Washington Suburban Sanitary Commission. Most of the analyses are performed at their water quality laboratories. The table inside this brochure shows the results of monitoring for the period of January 1st to December 31st, 2019.

LEAD AND COPPER TESTING - HOWARD COUNTY

Water is below detection levels when it leaves the water treatment plant for lead and copper, but lead and copper can be released when the water comes in contact with pipes and plumbing fixtures in homes and buildings that contain lead and/or copper. The EPA requires testing of the water distribution system for lead and copper at the tap. Howard County is required to sample 51 sites and of these 51 sites, 90% of the samples must have lead and copper levels less than the Action Level set by EPA, 0.015 mg/l or 15 parts per billion for lead and 1.3 mg/l or 1.3 parts per million for copper. The results of the sampling in 2014 are shown below. Howard County's lead and copper levels are consistently below the Action Level set by EPA. The next scheduled sampling for Lead and Copper will be performed during the summer of 2020. Check out our web page specific to lead in drinking water at: https://www.howardcountymd.gov/Departments/Public-Works/Bureau-Of-Utilities/Customer-Service-Division/Lead-in-Drinking-Water

Contaminant	Action Level	90th Percentile Value
Lead	15 ppb	0.11 ppb
Copper	1.3 ppm	0 ppm

If present, elevated levels of lead can cause serious health problems, especially for pregnant women and young children. Lead in drinking water is primarily from materials and components associated with service lines and home plumbing. Howard County's Bureau of Utilities is responsible for providing high quality drinking water, but cannot control the variety of materials used in private property plumbing components. When your water has been sitting for several hours, you can minimize the potential for lead exposure by flushing your tap for 30 seconds to 2 minutes before using water for drinking or cooking. If you are concerned about lead in your drinking water, you may wish to have your water tested. Information on lead in drinking water, testing methods and steps you can take to minimize exposure is available from the EPA Safe Drinking Water Hotline at 1-800-426-4791 or at http://water.epa.gov/drink/info/lead/**."**

Some people may be more vulnerable to contaminants in drinking water than the general population. Immunocompromised persons such as persons with cancer undergoing chemotherapy, persons who have undergone organ transplants, people with HIV/AIDS or other immune system disorders, some elderly, and infants can be particularly at risk from infections. These people should seek advice about drinking water from their health care providers. EPA/CDC guidelines on appropriate means to lessen the risk of infection by cryptosporidium and other microbiological contaminants are available from the Safe Drinking Water Hotline at 1-800-426-4791.

Waivers

The Maryland Department of the Environment has granted the City of Baltimore monitoring waivers for the following compounds: 2,3,7,8-TCDD (Dioxin), Endothall, Diquat,Glyphosphate, Asbestos and Cyanide.

Howard County Department of Public Works

Annual Water Quality Report

Reporting Period January 1, 2018 to December 31, 2018

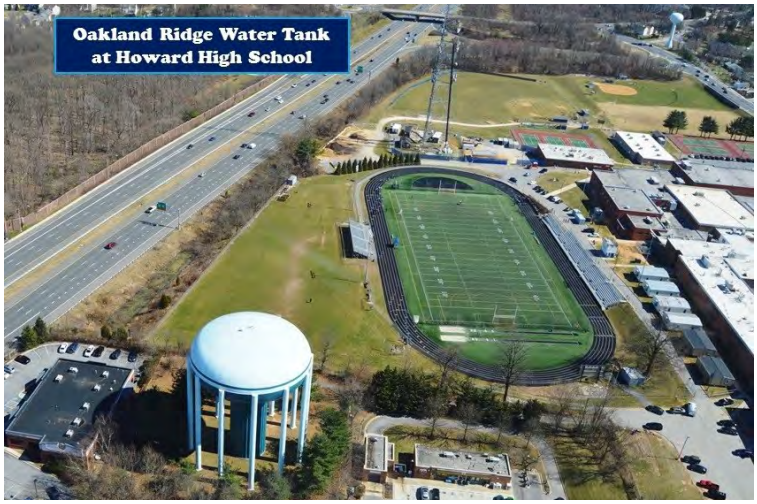
Calvin Ball,
Howard County Executive

Howard County Drinking Water

Access to clean water is a necessity and a human right. That is why we work hard to ensure that everyone in Howard County has access to quality drinking water. Our Bureau of Utilities is charged with conducting regular tests and publishing their results for the public.

This Consumer Confidence Report is a detailed summary of our community's drinking water quality. You will be able to learn more about how we make sure our water is clean and safe, and from where it is sourced.

I want to extend my thanks to every Howard County employee who works diligently in all types weather to protect our water quality and ensure uninterrupted service. They are the reason we have the most reliable water supply in the region so that we can all safely enjoy drinking from the tap.



Howard county is pleased to present to you this year's Water Quality Report. This report is designed to inform you about the quality water and services we deliver to you every day. Our constant goal is to provide you with a safe and dependable supply of drinking water. We want you to understand the efforts our water suppliers make to continually improve the water treatment process and protect our water resources. We are committed to ensuring the quality of your water. Our water sources are surface water from the Liberty Reservoir on the North Branch of the Patapsco River and the Loch Raven Reservoir on the main stream of the Gunpowder Fall purchased from Baltimore City and surface water from the Patuxent River purchased from the Washington Suburban Sanitary Commission

DEAR VALUED CUSTOMER,

Howard County residents, businesses, and guests continue to enjoy the highest quality drinking water in the region. In response to the moderate winter weather along with historic amount of rainfall experienced this past year by the region our motivated and well-trained staff were on continuous duty, promptly repairing broken water mains, and addressing damaged service lines. Our core responsibility is to proactively work each day to ensure critical water services are reliably provided on a 24/7 basis. Our mission is to provide high quality, safe and dependable drinking water to each of our valued customers. We hope you find this report informative and reassuring. In coordination with our regional water suppliers, the City of Baltimore and the Washington Suburban Sanitary Commission, we constantly strive to deliver the highest quality water supply service. The heightened national focus on the state of critical infrastructure is taken seriously and in Howard County our drinking water systems are expertly assessed for physical condition, proactively maintained to the highest standards, and considered for efficient rehabilitation or replacement in our long term capital improvement programming. Please do not hesitate in contacting your Howard County Bureau of Utilities team at 410-313-4900 for more information, or visit our updated web page at:
https://www.howardcountymd.gov/Departments/Public-Works/Bureau-Of-Utilities

***Art Shapiro*, PE, PMP**
Chief, Bureau of Utilities

"Reliable Professionals delivering customer-focused water services."

WHY WATER IS TESTED:

All sources of drinking water are subject to potential contamination by substances that are naturally occurring or manmade. These substances can be microbes, inorganic or organic chemicals and radioactive substances. As water travels over the land or underground, it can pick up substances or contaminants such as microbes, inorganic and organic chemicals, as well as radioactive substances, resulting from the presence of animals or from human activity. All drinking water, including bottled drinking water, may be reasonably expected to contain at least small amounts of some contaminants. The presence of contaminants does not necessarily indicate that the water poses a health risk.

Contaminants that may be present in source water include:

- Microbial contaminants, such as viruses and bacteria, which may come from sewage treatment plants, septic systems, agricultural livestock operations, and wildlife.
- Inorganic contaminants, such as salts and metals, which can be naturally occurring or result from urban storm water runoff, industrial or domestic wastewater discharges, oil and gas production, mining, or farming.
- Pesticides and herbicides, which may come from a variety of sources such as agriculture, urban storm water runoff, and residential uses.
- Organic chemical contaminants, including synthetic and volatile organic chemicals, which are by-products of industrial processes and petroleum production, and can also come from gas stations, urban storm water runoff, and septic systems.

To ensure that tap water is safe to drink, the Environmental Protection Agency (EPA) sets regulations that limit the amount of certain contaminants in water provided by public water systems. Food and Drug Administration (FDA) regulations set limits for contaminants in bottled water that must provide the same protection for public health.

The Maryland Department of the Environment (MDE) has completed a Source Water Assessment of the water supplies that serve the City of Baltimore. The Source Water Assessment Program may be viewed at the MDE web site, http://www.mde.state.md.us/programs/Water/Water_Supply/ConsumerConfidenceReports/Documents/CCR2015/Howard/0130002_Howard_County.pdf.

More information about contaminants and potential health effects can be obtained by calling the Environmental Protection Agency's Safe Drinking Water Hotline at 1-800-426-4791.

FOR MORE INFORMATION

If you have any questions about this report or concerning your water utility, please contact Howard County Utilities at 410-313-4900. We want our valued customers to be informed about their water utility. If you want to learn more, please attend any of our regularly scheduled Department of Public Works Board meetings. Please call 410-313-2330 for further information about these meetings.

Employees at Howard County Utilities work around the clock to provide top quality water to every tap. We ask that all our customers help us protect our water sources, which are the heart of our community, our way of life and our children's future.

TEST RESULTS – HOWARD COUNTY - PSWID 0130002

Contaminant	Violation Y/N	Total Sample Collected	Total Coliform* Positive	E-coli** Positive	E-coli MCLG	E-coli MCLG
Microbiological Contaminants						
Routine Samples	N	1804	8	0	0	0
Repeat Sample	N	24	0	0	0	0

*Coliform bacteria—naturally present in the environment
** E-coli—pathogen from human and animal fecal waste

TEST RESULTS – OUR SUPPLIERS

	Baltimore City Supply				Washington Suburban Sanitary Commission Supply				
	Ashburton Plant		Montebello Plant						
Contaminant - Units	Violation Y/N	Level Detected	Viola-tion Y/N	Level Detected	Violation Y/N	Level Detected	MCLG	MCL	Likely Source of Contamination
Microbiological Contaminants									
Turbidity - NTU	N	0.08	N	0.62	N	0.03	1.00	TT= Filtration	Soil runoff
Radioactive Contaminants									
Beta/photon emitters pCi/l	N	<1.5	N	<4	N	<4	0	50	Decay of natural and man-made deposits
Alpha emitters pCi/l	N	<1	N	<2	N	<2	0	15	Erosion of natural deposits
Inorganic Contaminants									
Antimony - ppb	N	<5	N	<5	N	ND	6	6	Discharge from petroleum refineries; fire retardants; ceramics; electronics; solder
Arsenic – ppb	N	<2	N	<2	N	ND	0	10	Erosion of natural deposits; runoff from orchards; runoff from glass And electronics production wastes
Barium – ppm	N	0.02	N	0.036	N	0.03	2	2	Discharge of drilling wastes; discharge from metal refineries; erosion of natural deposits
Beryllium – ppb	N	<0.5	N	<0.5	N	ND	4	4	Discharge from metal refineries And coal-burning factories; discharge from electrical, aerospace, And defense industries
Cadmium	N	<0.5	N	<0.5	N	ND	5	5	Erosion of natural deposits; runoff from orchards, runoff from glass & electronics production wastes
Chromium – ppb	N	<2	N	<2	N	ND	100	100	Discharge from steel and pulp mills; erosion of natural deposits
Copper – ppm	N	<.002	N	<.002	N	0.020	1.3	AL=1.3	Corrosion of household plumbing systems; erosion of natural deposits; leaching from wood preservatives
Fluoride – ppm	N	0.68	N	0.73	N	0.5	4	4	Erosion of natural deposits; water additive which promotes strong teeth; discharge from fertilizer and aluminum factories
Lead – ppb	N	<2	N	<2	N	ND	0	AL=15	Corrosion of household plumbing systems, erosion of natural deposits
Mercury (inorganic) Ppb	N	<0.5	N	<0.5	N	ND	2	2	Erosion of natural deposits; discharge from refineries and factories; runoff from landfills; runoff from cropland
Nitrate (as Nitrogen) Ppm	N	1.31	N	1.23	N	1.4	10	10	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits
Nitrite (as Nitrogen) Ppm	N	<0.01	N	<0.01	N	<0.05	1	1	Runoff from fertilizer use; leaching from septic tanks, sewage; erosion of natural deposits
Selenium – ppb	N	<5	N	<5	N	ND	50	50	Discharge from petroleum and metal refineries; erosion of natural deposits; discharge from mines
Thallium – ppb	N	<1	N	<1	N	ND	0.5	2	Leaching from ore-processing sites; discharge from electronics, glass, and drug factories
Synthetic Organic Contaminants including Pesticides and Herbicides									
2,4-D – ppb	N	<1.0	N	<1.0	N	ND	70	70	Runoff from herbicide used on row crops
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Atrazine – ppb	N	<3	N	<3	N	ND	3	3	Runoff from herbicide used on row crops
Benzo(a)pyrene – ppb	N	<0.2	N	<0.2	N	ND	0	0.2	Leaching from linings of water storage tanks and distribution lines
Carbofuran - ppb	N	<1.0	N	<1.0	N	ND	40	40	Leaching of soil fumigant used on rice and alfalfa
Chlordane - ppb	N	<2	N	<2	N	ND	0	2	Residue of banned termiticide
Dalapon – ppb	N	<4.0	N	<4.0	N	ND	200	200	Runoff from herbicide used on rights of way
Di(2-ethylhexyl) Adipate - ppb	N	<0.5	N	<0.5	N	ND	400	400	Discharge from chemical factories

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Dinoseb – ppb	N	<1.0	N	<1.0	N	ND	7	7	Runoff from herbicide used on soybeans and vegetables
Endrin – ppb	N	<0.5	N	<0.5	N	ND	2	2	Residue of banned insecticide
Ethylene dibromide - ppb	N	<0.05	N	<0.05	N	ND	0	0.05	Discharge from petroleum refineries
Heptachlor - ppb	N	<0.4	N	<0.4	N	ND	0	0.4	Residue of banned termiticide
Heptachlor epoxide - ppb	N	<0.2	N	<0.2	N	ND	0	0.2	Breakdown of heptachlor
Hexachlorobenzene - ppb	N	<0.5	N	<0.5	N	ND	0	1	Discharge from metal refineries and agricultural chemical factories
Hexachlorocyclo-pentadiene - ppb	N	<0.5	N	<0.5	N	ND	50	50	Discharge from chemical factories
Lindane-ppb	N	<0.2	N	<0.2	N	ND	0.2	0.2	Runoff/leaching from insecticide used on cattle, lumber, gardens
Methoxychlor - ppb	N	<0.5	N	<0.5	N	ND	40	40	Runoff/leaching from insecticide used on fruits, vegetables, alfalfa, livestock
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Picloram – ppb	N	<2.0	N	<2.0	N	ND	500	500	Herbicide runoff
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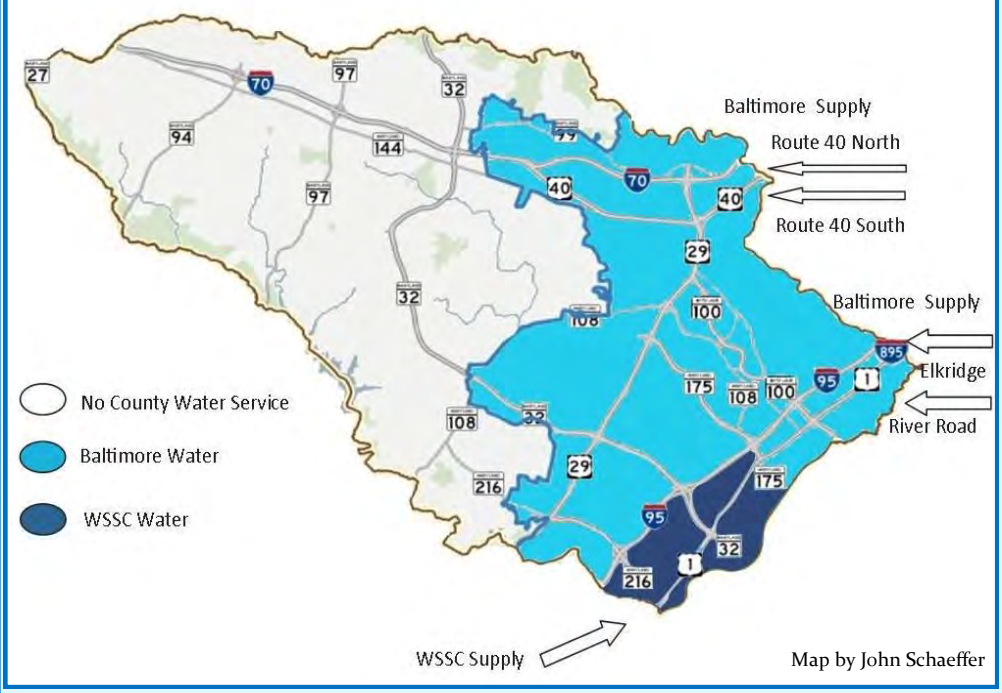
p-Dichlorobenzene - ppb	N	<0.5	N	<0.5	N	ND	75	75	Discharge from industrial chemical factories
1,2 – Dichloroethane - ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from industrial chemical factories
1,1 – Dichloroethane - ppb	N	<0.5	N	<0.5	N	ND	7	7	Discharge from industrial chemical factories
cis-1,2-Dichloroethene - ppb	N	<0.5	N	<0.5	N	ND	70	70	Discharge from industrial chemical Factories
trans-1,2 Dichloroethene - ppb	N	<0.5	N	<0.5	N	ND	100	100	Discharge from industrial chemical factories
Dichloromethane– ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from pharmaceutical and chemical factories
1,2-Dichloropropane Ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from industrial chemical factories
Ethylbenzene – ppb	N	<0.5	N	<0.5	N	ND	700	700	Discharge from petroleum refineries
Haloacetic Acids, Total– ppb	N	42.0	N	37.0	N	53	0	60	By-product of drinking water chlorination
Styrene – ppb	N	<0.5	N	<0.5	N	ND	100	100	Discharge from rubber and plastic factories; leaching from landfills
Tetrachloroethylene – ppb	N	<0.5	N	<0.5	N	ND	0	5	Leaching from PVC pipes; discharge from factories and dry cleaners
1,2,4-Trichlorobenzene - Ppb	N	<0.5	N	<0.5	N	ND	70	70	Discharge from textile-finishing factories
1,1,1 – Trichloroethane - Ppb	N	<0.5	N	<0.5	N	ND	200	200	Discharge from metal degreasing sites and other factories
1,1,2 –Trichloroethane - Ppb	N	<0.5	N	<0.5	N	ND	3	5	Discharge from industrial chemical factories
Trichloroethene – ppb	N	<0.5	N	<0.5	N	ND	0	5	Discharge from metal degreasing sites and other factories
TTHM - ppb [Total trihalomethanes]	N	48.0	N	53.0	N	66	0	80	By-product of drinking water chlorination
Vinyl Chloride - ppb	N	<0.5	N	<0.5	N	ND	0	2	Leaching from PVC piping; discharge from plastics factories
Toluene – ppb	N	<0.5	N	<0.5	N	ND	1000	1000	Discharge from petroleum factories
Xylenes – ppb	N	<0.5	N	<0.5	N	ND	10000	10000	Discharge from petroleum factories; discharge from chemical factories

TEST RESULTS - HOWARD COUNTY—PSWID 0130002						
Volatile Organic Chemicals						
Substance	MCLG	MCL	Range (LRAA)	Average	Violation	Major Sources
Total THM's	n/a	**80ppb**	27.4 - 99.2ppb	47ppb	No	Byproduct of drinking water chlorination
HAA(5)	n/a	**60ppb**	23.9 - 45.9 ppb	34ppb	No	Byproduct of drinking water chlorination



WHERE YOUR WATER COMES FROM

If you live in the North Laurel area, east of Interstate 95 and south of Patuxent Range Road, your water originates from the Washington Suburban Sanitary Commission in Laurel. If you live anywhere else in Howard County and are connected to the public water supply, your water originates from Baltimore City. As a "Consecutive Water System", Howard County purchases water from Baltimore City and the Washington Suburban Sanitary Commission. Most of the analyses are performed at their water quality laboratories. The table inside this brochure shows the results of monitoring for the period of January 1st to December 31st, 2016.

LEAD AND COPPER TESTING - HOWARD COUNTY

Water is below detection levels when it leaves the water treatment plant for lead and copper, but lead and copper can be released when the water comes in contact with pipes and plumbing fixtures in homes and buildings that contain lead and/or copper. The USEPA requires testing of the water distribution system for lead and copper at the tap. Howard County is required to sample 51 sites and of these 51 sites, 90% of the samples must have lead and copper levels less than the Action Level set by EPA, 0.015 mg/l or 15 parts per billion for lead and 1.3 mg/l or 1.3 parts per million for copper. The results of the sampling in 2014 are shown below. Howard County's lead and copper levels are consistently below the Action Level set by EPA. The next scheduled sampling for Lead and Copper will be performed during the summer of 2020. Check out our web page specific to lead in drinking water at: https://www.howardcountymd.gov/Departments/Public-Works/Bureau-Of-Utilities/Customer-Service-Division/Lead-in-Drinking-Water

Contaminant	Action Level	90th Percentile Value
Lead	15 ppb	0.11 ppb
Copper	1.3 ppm	0 ppm

If present, elevated levels of lead can cause serious health problems, especially for pregnant women and young children. Lead in drinking water is primarily from materials and components associated with service lines and home plumbing. Howard County's Bureau of Utilities is responsible for providing high quality drinking water, but cannot control the variety of materials used in plumbing components. When your water has been sitting for several hours, you can minimize the potential for lead exposure by flushing your tap for 30 seconds to 2 minutes before using water for drinking or cooking. If you are concerned about lead in your drinking water, you may wish to have your water tested. Information on lead in drinking water, testing methods, and steps you can take to minimize exposure is available from the EPA Safe Drinking Water Hotline at 1-800-426-4791 or at http://water.epa.gov/drink/info/lead/**."**

Some people may be more vulnerable to contaminants in drinking water than the general population. Immuno-compromised persons such as persons with cancer undergoing chemotherapy, persons who have undergone organ transplants, people with HIV/AIDS or other immune system disorders, some elderly, and infants can be particularly at risk from infections. These people should seek advice about drinking water from their health care providers. EPA/CDC guidelines on appropriate means to lessen the risk of infection by cryptosporidium and other microbiological contaminants are available from the Safe Drinking Water Hotline at 1-800-426-4791.

Waivers

The Maryland Department of the Environment has granted the City of Baltimore monitoring waivers for the following compounds: 2,3,7,8-TCDD (Dioxin), Endothall, Diquat,Glyphosphate, Asbestos and Cyanide.

From: Alexander Sulakvelidze
To: Hice, Stephanie
Subject: [EXTERNAL] RE: GRN 000966 - Questions for Notifier (USDA/FSIS)
Date: Friday, March 5, 2021 3:41:42 PM
Attachments: image001.png
Response to FDA _ March 5 2021.pdf

Dear Stephanie,

Attached please find our responses to the FSIS USDA questions regarding our GRAS Notice No. 000966. Please let me know if you have any additional questions or comments regarding this application.

Thank you!

Sandro

Alexander Sulakvelidze, Ph.D.
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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Wednesday, February 24, 2021 3:51 PM
To: Alexander Sulakvelidze <asulakvelidze@intralytix.com>
Subject: RE: GRN 000966 - Questions for Notifier (USDA/FSIS)

Dear Dr. Sulakvelidze,

During review of GRAS Notice No. 000966, USDA/FSIS noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include

any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to USDA/FSIS's comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Response to Questions/Comments Regarding GRN 000966 from USDA FSIS

1. All of the studies (veal, chicken tenderloin, and ground chicken) were conducted on product held at 10°C, or 50°F. Using ground chicken as an example, the data shows approximately a .5 log reduction in C. jejuni after 6 hrs. with no significant improvement with longer hold times. However, poultry is often kept at 40 or below. How does a lower product temperature effect the results shown in the tables in Appendix 1.3?

Re. Bacteriophages retain their activity at a wide range of temperatures including 40°F (4°C) and, in general, demonstrate similar efficacy at chill temperature (4°C) and room temperature. This has been well established for various bacteriophages. For example, a study by the USDA investigators[1] demonstrated that *E. coli* O157:H7 specific phage preparation ECP-100 (currently trade named as EcoShield™) showed a similar or improved efficacy at 4°C compared to 20°C. Another study[2] found that a *Salmonella*-specific phage preparation showed a higher efficacy at 4°C vs 22°C. Similar observations have been also made for *Campylobacter* phages. For example, Bigwood et. al.[3] found that reductions in *C. jejuni* levels were similar when meat samples treated with *Campylobacter* phages were stored at 4°C and 24°C. Therefore, we expect that the CampyShield™ phages will show a similar efficacy at 4°C compared to what we demonstrated in our studies submitted to the FSIS when foods were stored at 10°C.

The studies submitted to the FSIS were conducted at 10°C to mimic a moderate temperature abuse situation that may occur during processing and supply chain. Although processors make every effort to maintain the recommended storage and processing temperatures, temperature variations/abuses do occur and jeopardize the safety of the food products [4,5]. Our studies demonstrate that even under these temperature abuse conditions (i.e., product warming) CampyShield™ will provide effective control of *Campylobacter* contamination.

2. The Campylobacter reduction on chicken tenders averaged greater than 0.81 logs. As tenders have little surface fat, does lean/fat ratio affect the efficacy of the phages? Does the company have data to support the phage's efficacy on other types of poultry products (specifically skin-on poultry products)?

Re. Several published studies have shown that the lean/fat ratio of foods does not materially affect the efficacy of bacteriophage treatment[6,7,8]. For example, EcoShield™ PX (GRN 834) is efficacious on a wide variety of food products with a broad range of lean/fat ratio. These products include beef, ground beef, raw

---

[1] Sharma M., Patel J.R., Conway W.S., Ferguson S. and Sulakvelidze A. Effectiveness of bacteriophages in reducing *Escherichia coli* O157:H7 on fresh-cut cantaloupes and lettuce. J Food Prot 2009; 72:1481-5.

[2] Sharma M., Dashiell G., Handy E.T., East C., Reynnells R., White C., Nyarko E., Micallef S., Hashem F. and Millner P.D. Survival of *Salmonella* Newport on whole and fresh-cut cucumbers treated with lytic bacteriophages. J. Food Prot. 2017; 80:668-673.

[3] Bigwood T., Hudson J.A., Billington C., Carey-Smith G.V. and Heinemann J.A. Phage inactivation of foodborne pathogens on cooked and raw meat. Food Microbiol 2008; 25:400-6.

[4] Ingham S.C., Losinski J.A., Becker K.L. and Buege D.R. Growth of *Escherichia coli* O157:H7 and *Salmonella* serovars on raw beef, pork, chicken, bratwurst and cured corned beef: Implications for HACCP plan critical limits. Journal of Food Safety 2004; 24:246-256.

[5] Russell S.M., Fletcher D.L. and Cox N.A. The Effect of Temperature Mishandling at Various Times During Storage on Detection of Temperature Abuse of Fresh Broiler Chicken Carcasses. Poultry Science 1996; 75:261-264.

[6] Vikram A., Tokman J., Woolston J. and Sulakvelidze A. Phage biocontrol improves food safety by significantly reducing both the concentration and occurrence of *Escherichia coli* O157:H7 in various foods. J Food Prot 2020; 83:668-676.

[7]Vikram A., Woolston J. and Sulakvelidze A. Phage Biocontrol Applications in Food Production and Processing. Curr. Issues Mol. Biol. 2021; 40:267-302.

[8] Coffey B., Mills S., Coffey A., McAuliffe O. and Ross R.P. Phage and their lysins as biocontrol agents for food safety applications. Annu Rev Food Sci Technol 2010; 1:449-68.

chicken breast, ground chicken, and fish. The fat content of these foods varies from 0.5% for poultry products to over 15% for fish products. Similarly, CampyShield™ is effective on red meat, poultry, and ground poultry which typically vary in their fat:protein ratio from ca. 0.5% to ca. 8% fat content.

The ground chicken study included with our GRAS notification showed similar efficacy on foods with various fat content: ground chicken has a higher fat content (ca. 8%) than chicken tenderloins (ca. 0.5%). Therefore, we expect that CampyShield™ will be effective on all types of poultry products, including skin-on products. Moreover, and directly pertinent to our CampyShield™ preparation, several studies with other but technically equivalent phage preparations demonstrated that *Campylobacter* phages could be very effective in reducing *Campylobacter* levels on skin-on poultry products. For example, application of 6.0-7.0 log PFU/cm^2 of lytic phages was reported to reduce *Campylobacter jejuni* population on chicken skin by 1.0-1.3 log[9,10]. The lytic phages in CampyShield™ are functionally equivalent to the lytic phages used in those studies and we fully expect them to reduce *C. jejuni* contamination on all poultry products, including chicken skin.

3. With regard to the study evaluating efficacy in ground product, please identify the total amount of liquid added to the ground product through the addition of the phage solution on a percentage basis.

Re. In the referenced study CS20G20MCB, 2.86 mL of CampyShield™ or PBS was sprayed on 325 g of ground poultry. Therefore, the total amount of liquid added is $\left(\frac{2.86}{325}\right) x100 = 0.88\%$.

4. When considering in-plant conditions, specifically, post chill further processing typically does not happen at room temperature and every effort is made to maintain a chilled product temperature. The study presents product that is held at room temperature for 20 minutes, so we could not determine if a difference in temperature would affect the activity of the phage. What effect would be expected on the efficacy of phage from a lowered temperature in these applications?

Re. In all studies submitted to the FSIS, the food products were stored refrigerated at 4°C prior to testing, to prevent spoilage. When the test started, the products were removed from the refrigerator and experimentally contaminated with the challenge *Campylobacter* strain. After contamination, foods were held at room temperature for 20 min to enable attachment of the *C. jejuni* to the food products, then treated with phages (or PBS in the control group), and immediately returned to refrigerated storage. The *C. jejuni* challenge and subsequent phage applications were performed in a Biosafety Cabinet, therefore at room temperature, for safety reasons. This method of inoculation is fairly routine in studies monitoring foodborne pathogens, including *C. jejuni* [9,11]. Furthermore, the 20-minute period is a trivial amount of time, especially when compared to the overall study duration of 48 h or 2,880 minutes. Moreover, and as explained in more detail in our response to Question #1 above, efficacy of phages (including CampyShield™) in reducing the levels of their targeted bacteria in various foods is similar across a range of temperatures; therefore, holding food products at room temperature for a short period of time (20 min) is not expected to impact CampyShield™ efficacy in any meaningful manner.

---

[9] Goode D., Allen V.M. and Barrow P.A. Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. Appl. Environ. Microbiol. 2003; 69:5032-5036.
[10] Atterbury R.J., Connerton P.L., Dodd C.E., Rees C.E. and Connerton I.F. Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. Appl Environ Microbiol 2003; 69:6302-6.
[11] Zhao T. and Doyle M.P. Reduction of Campylobacter jejuni on Chicken Wings by Chemical Treatments. Journal of Food Protection 2006; 69:762-767.

5. The studies provided show that there is a complete suppression of microbial growth at 48 hours. However, in order to support a processing aid determination, we would need to see more data points over time sufficient to show a lag phase and regrowth.

Re. It will be very difficult, if not impossible, to show a significant regrowth of *Campylobacter* under the study conditions because *C. jejuni* does not grow/multiply at temperatures below 30°C (but they can survive and retain infectivity [12,13]). Noteworthy, in all the studies submitted with our GRAS application, no growth of *C. jejuni* was observed in any of the control samples either (i.e., samples not treated with phages). Therefore, the observation that there was no regrowth of the bacterium in phage treated samples after the initial reduction in *Campylobacter* levels is not an indication of continued technical effect. As noted above, there was no regrowth in control (phage-untreated) samples either, indicating that *Campylobacter* simply did not continue to grow under the study conditions (i.e., temperature of ~10°C).

We believe CampyShield™ meets the processing aid designation as defined in part "**c**" of the Food and Drug Administration's regulations (21 CFR 101.100 (a) (3) (ii)): "**c**. *Substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food."* For example, and as correctly noted by the FSIS reviewer(s) in Question #1 above, the data submitted to the FSIS showed that after the initial reduction in *Campylobacter* levels at 6 h, CampyShield™ did not exhibit any continued technical effect as it did not reduce further the levels of the bacteria throughout the 48 h testing duration. Moreover, the recontamination study (#CS20G22MC) submitted to the FSIS also showed that CampyShield™ did not exert a continued technical effect. In that study, the ground chicken samples were contaminated and treated with CampyShield™ and subsequently re-contaminated with *C. jejuni* at 48 h. CampyShield™ did not reduce *C. jejuni* levels on the re-contaminated products indicating that there was no continued technical or functional effect in those foods. The lack of continued technical effect is not specific or limited to CampyShield™ but rather is characteristic to all other phage preparations used for food safety applications. Indeed, several other technically equivalent GRAS-cleared phage preparations for food safety applications (e.g., GRN 917, GRN 834, GRN 827, and GRN 435) also have been granted the processing aid designation.

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[12] Park S.F. The physiology of Campylobacter species and its relevance to their role as foodborne pathogens. International Journal of Food Microbiology 2002; 74:177-188.
[13] Solow B.T., Cloak O.M. and Fratamico P.M. Effect of Temperature on Viability of Campylobacter jejuni and Campylobacter coli on Raw Chicken or Pork Skin†. Journal of Food Protection 2003; 66:2023-2031.

From: Alexander Sulakvelidze
To: Hice, Stephanie
Subject: [EXTERNAL] RE: GRN 000966 - Questions for Notifier
Date: Friday, April 30, 2021 5:04:10 PM
Attachments: image001.png
Response to FDA_additional information_April 30 2021.pdf

Dear Stephanie,

Please see attached summary of the additional information regarding GRN 000966. We promised to provide this information in our February 4, 2021 response to the FDA.

Please let me know if you have any further questions regarding the additional information provided, or if any of our answers requires further clarification.

We will be responding to the remaining question we received for this GRAS notice on 4/28/21, by the end of next week.

Thank you!

Sandro Sulakvelidze

Alexander Sulakvelidze, Ph.D.
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From: Alexander Sulakvelidze
Sent: Thursday, February 4, 2021 3:01 PM
To: 'Hice, Stephanie' <Stephanie.Hice@fda.hhs.gov>
Subject: RE: GRN 000966 - Questions for Notifier

Dear Stephanie,

Please see attached our responses to the FDA questions of January 26, 2021. As you will see in our responses, we will be doing some additional testing (e.g., for lead) and will provide the results to the FDA when they are available.

In the meantime, please let me know if you have any additional questions or if any of our answers requires further clarification.

Thank you!

Sandro Sulakvelidze

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Tuesday, January 26, 2021 8:07 AM
To: Alexander Sulakvelidze <asulakvelidze@intralytix.com>
Subject: GRN 000966 - Questions for Notifier

Dear Dr. Sulakvelidze,

During our review of GRAS Notice No. 000966, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
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Additional Information

In response to the FDA questions of January 26, 2021 regarding GRN #000966

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Additional Information

(in response to the FDA questions of January 26, 2021)

In our response to the FDA on 2/4/21, we promised to provide additional information regarding Questions 7, 8, and 10 posed by the FDA in communication on 1/26/2021. We were also able to obtain additional information regarding Question 3.

FDA Question # 3 (1/26/21)

In our response to Question 3, we stated that the three phages included in the current version of CampyShield™ had been deposited with the American Type Culture Collection (ATCC), but we had not yet received the patent depository numbers as of 2/4/21. Those numbers are now available, please see below (Table 1).

Table 1 List of current CampyShield monophages and their respective ATCC patent depository numbers

Phage Designation	**ATCC #**
CJLB-5 (J350)	PTA-126840
CJLB-10 (J375)	PTA-126842
CJLB-14 (J386)	PTA-126845

FDA Question # 7 (1/26/21)

In Question 7, the FDA requested we discuss why our raw materials did not pose a safety concern due to major allergens that might be present in the raw materials used in the fermentation. In our response to Question 7, we explained why CampyShield™ is free from all known major allergens that may be contributed by the media (i.e., soy and wheat allergens).[1] Nevertheless, we committed

[1] Briefly: (i) the media manufacturer uses hydrolyzed ingredients for media preparation (which significantly reduces the allergenicity of the soy and wheat proteins), (ii) the host cell culture further hydrolyzes the media components, including the soy- and wheat-based peptones, and (iii) the extensive filtration and washing of CampyShield™ monophages would remove any remaining allergens, if present.

to the FDA that we would test three non-consecutive lots of CampyShield™ for the presence of soy and wheat allergens. This testing, of four non-consecutive lots, has been completed. In addition, we also tested the vegan media used for production of the component phages, VIB (Vegitone Infusion Broth), for the presence of soy and wheat allergens. This testing has been also completed. No soy allergens and no wheat allergens have been detected in any of the samples analyzed, including the four recently prepared non-consecutive lots of CampyShield™. It should be noted that VIB was expected to be free of soy and wheat allergens as it contains the hydrolyzed proteins. The current testing was performed to empirically demonstrate that the media does not contain the allergens. Since the media is the only potential source of allergens, these confirmatory tests in combination with our rigorous filtration process (as demonstrated by absence of the allergens in four non-consecutive CampyShield™ lots) unambiguously establish that CampyShield™ preparations are free of the allergens. The results of this testing are presented in Table 3.

FDA Question # 8 (1/26/21)

In Question 8, the FDA requested a specification for lead be included in Table 2, page 5 of the original GRAS notice GRN #000966. In our response to Question 8, we conducted a risk assessment regarding the presence of lead in CampyShield™ and concluded that the risk for introduction of lead into the product or process is extremely low, if not non-existing. Therefore, we do not believe that including specification for lead for commercial production lots of CampyShield™ is warranted. We did commit to (i) testing at least three non-consecutive lots of CampyShield™ for the presence of lead, and (2) continued regular testing of our water (as the potential source of lead) and notifying the FDA should lead be detected at any time in the future. Testing for lead has been completed for four non-consecutive lots of CampyShield™. In addition, we also tested the VIB media and PBS (phosphate buffered saline), the buffer in which the CampyShield™ cocktail is blended, for lead. No traces of lead have been detected in any of the samples analyzed, including the four recently prepared non-consecutive lots of CampyShield™. The results of this testing are presented in Table 3. As mentioned previously, we will continue to monitor lead levels in the incoming water and will notify the FDA if lead is detected above the maximum EPA-established allowable level of 15 µg/L for drinking water.

FDA Question # 10 (1/26/21)

In Question 10, the FDA requested that we provided results from a minimum of three (preferably five) non-consecutive batches to demonstrate that the phage preparation can be manufactured to meet the provided specifications listed in Table 2. As indicated in our previous response to Question 10, the GRAS notification included three *consecutive* lots of CampyShield™. As per the FDA request, we prepared an additional four *non-consecutive* lots of CampyShield™ and analyzed them for the compliance with the specifications provided on page 5 of our original GRAS notice GRN #000966. All these recently prepared non-consecutive batches of CampyShield™ met all specifications set forth in our GRAS notice. The new testing results are shown in Table 2.

Table 2 QC results for four non-consecutive lots of CampyShield™

QC Specification	CampyShield™ Specification	0421D1510A28	0421D2210B26	0421D2310B44	0421D2310D60
Potency (PFU/mL)*	10.0±0.33 $\log_{10}$ PFU/mL (4.68E9 – 2.14E10)	9.8 $\log_{10}$	9.9 $\log_{10}$	10.0 $\log_{10}$	10.1 $\log_{10}$
Microbial purity	No growth	No growth	No growth	No growth	No growth
Endotoxin Content (EU/mL)	≤ 25,000 EU/mL (at ~9.0 $\log_{10}$ PFU/mL)	13,290	3,843	5,016	3,256
Identity	All phages included	All 3 component monophages present.	All 3 component monophages present.	All 3 component monophages present.	All 3 component monophages present.
Specifications met? Yes / No		Yes	Yes	Yes	Yes

* CampyShield™ will be offered in various concentrations, such as 10X, 30X, or 50X and appropriate dilution / use instructions will be provided; these four lots were prepared at the 10X concentration (i.e., 10.0 $\log_{10}$ PFU/mL). The error range of the phage titration / potency test is 0.33 $\log_{10}$ [2].

Table 3 Analysis of four non-consecutive lots of CampyShield™, the propagation media, and storage buffer

Additional testing	0421D1510A28	0421D2210B26	0421D2310B44	0421D2310D60	VIB	PBS
Soy allergen	ND	ND	ND	ND	ND	NT
Wheat allergen	ND	ND	ND	ND	ND	NT
Lead	ND	ND	ND	ND	ND	ND

ND = Not detected; NT = Not tested

[2] Anderson, B, MH Rashid, C Carter, G Pasternack, C Rajanna, T Revazishvili, T Dean, A Senecal, and A Sulakvelidze. 2011. "Enumeration of bacteriophage particles: Comparative analysis of the traditional plaque assay and real-time QPCR- and nanosight-based assays." Bacteriophage 1 (2):86-93.

From: Alexander Sulakvelidze
To: Hice, Stephanie
Subject: [EXTERNAL] RE: GRN 000966 - Questions for Notifier (USDA/FSIS)
Date: Wednesday, May 12, 2021 2:26:10 PM
Attachments: image001.png
Response to the FDA w Supporting Materials _ May 12 2021.pdf

Dear Stephanie,

Attached is our response to the additional request and comment posed by the FSIS USDA on 5/10/21, regarding GRN 000966.

Please let me know if you have any further questions or if any of our answers requires further clarification.

On a different subject: any further word on the Supplement to the GRAS Notice 435 for SalmoFresh? As you may recall, the supplement merely requested increase in application rate of SalmoFresh from 10^7 PFU/g to 10^8 PFU/g. We have several customers that feel this product can help improve the safety of their foods and we are all waiting for the FDA clearance to proceed with the application.

Thank you!

Sandro

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Monday, May 10, 2021 8:29 AM
To: Alexander Sulakvelidze <asulakvelidze@intralytix.com>
Subject: RE: GRN 000966 - Questions for Notifier (USDA/FSIS)

Dear Dr. Sulakvelidze,

During review of GRAS Notice No. 000966, USDA/FSIS noted an additional question and a comment that needs to be addressed and is attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in

advance for your attention to USDA/FSIS's comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Regulatory Review Scientist & Microbiology Reviewer
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Theirs (what is this?)

Additional Information

In response to the FDA questions of May 10, 2021 regarding GRN #000966

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Questions/Comments Regarding GRN 000966 from USDA FSIS (May 10, 2021):

1. ***Can the company provide an SDS?***

The SDS is attached.

2. ***FSIS Comment***

"The company indicated that they used 2.86 mL of CampyShield™ or PBS sprayed on 325 g of ground poultry. Therefore, the total amount of liquid added is 2.86/325 xx100 = 0.88%. FSIS Response: We are currently working with our leadership regarding our policy on adding phage directly to ground product. So, in this case (also considering the amount of liquid being added here), we would have to require descriptive labeling for this in ground product. At this current time, it would be listed with descriptive labeling consistent with current policy, under the understanding that the listing might be updated if their support aligns with the requirements of any future policy changes."

CampyShield™ is intended to be used as an antimicrobial processing aid on (i) raw and ground poultry and (ii) raw red meat products. In other words, for the ground products, we are only requesting application of CampyShield in ground poultry and not in ground red meat. We understand that the FSIS is currently reviewing the labeling policy with regards to phage applications on ground red meat. That application is more relevant for other phage preparations (e.g., EcoShield PX) and we hope that the FSIS will allow such applications in the near future for the products that include treating ground red meat in their intended uses. However, since GRN000966 does not stipulate application of CampyShield on ground red meat, we do not believe labeling requirement should apply for the intended use under the current policy.

In this context, in the FSIS Directive 7120.1, several bacteriophage preparations are approved for use in ground products without labelling. For example, GRN468 is for "ground red meat", GRN603 is for "poultry" in general (thus presumably including ground poultry as well), and GRN435 is for "ground poultry". The most recent version of Directive 7120.1 has inadvertently removed ground poultry from listing of our product SalmoFresh™ (GRN 435) which we believe is currently being restored; in the meantime, FSIS has confirmed on at least two separate occasions that SalmoFresh™ is approved for use in ground poultry with no labeling requirement. Please see attached documents. In the file *Supporting_document_1*, FSIS confirms that SalmoFresh™ (GRN 435) is approved "for use in poultry (to include raw poultry prior to and after grinding)". Additional correspondence from FSIS (see *Supporting_document_2*) confirms that the establishment which has been using SalmoFresh™ in their ground poultry products with no labeling may continue such use (see highlighted text). Therefore, we believe no labeling requirement should also apply to CampyShield, for its intended use – which includes (i) raw and ground poultry and (ii) raw red meat products, *but not ground red meat*.

CampyShield™
Campylobacter-specific phage preparation

Section 1: Identification

Product identifier

Product name: CampyShield™
Catalog #: 04CP

Recommended use of the chemical and restrictions on use

Phage preparation effective against Campylobacter spp.

Supplier's details

Intralytix, Inc.
8681 Robert Fulton Dr.
Columbia, MD 21046

Emergency phone number

1-877-ITX-PHAGE
Monday–Friday 9:00 AM – 5:00 PM

Section 2: Hazard identification

Classification of substance or mixture

Not a hazardous substance or mixture

GHS label elements, including precautionary statements

Not a hazardous substance or mixture

Other hazards which do not result in classification

None

Section 3: Composition/information on ingredients

Mixture

Bacteriophages in aqueous 0.1M sodium chloride solution

Component list

Component	% composition	CAS #	Classification
Water	> 99.0	7732-18-5	Not applicable
Sodium chloride	0.91	7647-14-5	Not applicable
Potassium phosphate monobasic	0.01	7778-77-0	Not applicable
Sodium phosphate dibasic	0.04	7558-79-4	Not applicable

Campylobacter – specific phages	< 0.01	Not applicable	Not applicable

Section 4: First aid measures

Description of first-aid measures

If inhaled:

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact:

Wash off with soap and water.

In case of eye contact:

Flush eyes with water as a precaution.

If swallowed:

Never give anything by mouth to an unconscious person. If swallowed in excess, rinse mouth with water as a precaution.

Most important symptoms and effects, both acute and delayed:

The most important known symptoms and effects are described in the labelling (see Section 2) and/or in Section 11.

Indication of any immediate medical attention and special treatment needed:

No data available

Section 5: Fire fighting measures

Suitable extinguishing media

No restrictions

Specific hazards arising from the chemical

None

Special protective actions for fire-fighters

None

ection 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures

For personal protection, see Section 8.

Environmental precautions

No special environmental precautions required

Method and materials for containment and cleaning up

Keep in suitable closed containers. Mop up or absorb with an inert dry material and place in an appropriate waste disposal container. No specific spill kit is required for this product

Section 7: Handling and storage

Precautions for safe handling

For precautions, see Section 2

Conditions for safe storage, including any incompatibilities

Keep container closed, refrigerated at 2-8°C, and protected from light.

ection 8: Exposure controls / personal protection

Control parameters

Contains no substances with occupational exposure limit values.

Appropriate engineering controls

General industrial hygiene practice

Individual protection measures, such as personal protective equipment (PPE)

Eye/face protection

When using as an aerosol, wear eye protection and provide access to eye/face flushing equipment.

Skin protection

A lab coat and/or gloves may be worn when handling this solution.

Respiratory protection

When airborne exposure limits are exceeded or ventilation is inadequate, use appropriate NIOSH approved respiratory protection equipment. Respiratory protection programs are subject to 29 CFR § 1910.134.

Section 9: Physical and chemical properties

Information on basic physical and chemical properties

Appearance	Clear/opalescent liquid
Odor	None
Odor threshold	No data available
pH	7.3 – 7.5
Melting point / freezing point	May start to solidify at –0.1°C (31.8°F) (WATER)
Initial boiling point and boiling range	The lowest known value is 99.9°C (211.8°F) (WATER).
Flash point	No data available
Evaporation rate	No data available
Flammability	No data available
Upper/lower flammability or explosive limits	No data available
Vapor pressure	No data available

Vapor density	No data available
Relative density	1.01 g/cm^3
Solubility	Soluble in water
Partition coefficient: n-octanol/water	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available

Section 10: Stability and reactivity

Reactivity

No data available

Chemical stability

Stable under recommended storage conditions

Possibility of hazardous reactions

No data available

Conditions to avoid

No data available

Incompatible materials

No data available

Hazardous decomposition products

No data available

Section 11: Toxicological information

Acute toxicity

No evidence of acute toxicity

Skin corrosion/irritation

Conclusive but not sufficient for classification

Serious eye damage/irritation

No data available

Respiratory or skin sensitization

Conclusive but not sufficient for classification

Mutagenicity

Conclusive but not sufficient for classification

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probably, possible, or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity

Conclusive but not sufficient for classification

STOT-single exposure

Conclusive but not sufficient for classification

STOT-repeated exposure

Conclusive but not sufficient for classification

Aspiration hazard

Conclusive but not sufficient for classification

Section 12: Ecological information

Toxicity

No data available

Persistence and degradability

No data available

Bioaccumulative potential

No data available

Mobility in soil

No data available

Other adverse effects

No data available

Section 13: Disposal considerations

Disposal methods

Product

Material does not have an EPA Waste Number and is not a listed waste, however, always contact a permitted waste disposal (TSD) to assure compliance with all current local, state, and Federal Regulations.

Packaging

Package may be recycled, if such disposal options exist.

Section 14: Transport information

UN Number

Not relevant

UN Proper Shipping Name

Not relevant

Transport hazard class

Not hazardous

Packing group

Not relevant

Environmental hazards

Not relevant

Special precautions

Keep refrigerated / cool during shipment

Section 15: Regulatory information

TSCA

Not applicable

SARA 302

Not applicable

SARA 311/312

Not applicable

SARA 313

Not applicable

CERCLA

Not applicable

California Proposition 65

This product does not contain any Proposition 65 chemicals.

US State Right-to-Know Regulations

Not applicable

Section 16: Other information

Revision date
May 10, 2021

Version
1

Further information
Notice to Reader

The statements contained herein are based upon technical data that Intralytix, Inc. believes to be reliable, are offered for information purposes only and as a guide to the appropriate precautionary and emergency handling of the material by a properly trained person having the necessary technical skills. Users should consider these data only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use, storage and disposal of these materials and the safety and health of employees and customers and the protection of the environment.

INTRALYTIX, INC. MAKES NO REPRESENTATION OR WARRANTY OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE, WITH RESPECT TO THE INFORMATION HEREIN OR THE PRODUCT TO WHICH THE INFORMATION REFERS.

Supporting_document_1

Correspondence from Dr. Rachel Edelstein, Office of Policy and Program Development, Food Safety and Inspection Service, USDA, regarding GRN 435 (SalmoFresh)

From: "Edelstein, Rachel - FSIS" <rachel.edelstein@usda.gov>
Date: February 12, 2021 at 2:14:38 PM EST
To: Lisa Wallenda Picard <lpicard@turkeyfed.org>
Cc: "Murphy-Jenkins, Rosalyn - FSIS" <rosalyn.murphy-jenkins@usda.gov>, "Canavan, Jeff - FSIS" <jeff.canavan@usda.gov>, "Carter, Melvin - FSIS" <melvin.carter@usda.gov>, "Hretz, Stephanie - FSIS" <stephanie.hretz@usda.gov>
Subject: RE: Thank you

Hi Lisa—As you and I just discussed, with regard to the information for GRN 435 (SalmoFresh) that you sent, we have confirmed that it was evaluated and approved for use in poultry (to include raw poultry prior to and after grinding) applied as a spray up to 10^7 plaque forming units (pfu) per gram of food product without labeling. I am sorry that we missed this one earlier!

Our contact at FDA is Dr. Rachel Morissette with the Division of Food Ingredients at FDAs Center for Food Safety and Applied Nutrition (CFSAN). Her email address is rachel.morissette@fda.hhs.gov. She will be able to provide information on how a phage company/manufacturer can set up a pre-meeting with subject matter experts in their group to determine what may be needed to proceed with submitting a new GRAS notice or supplementing an existing one. As discussed, many of the phage listings currently listed on Directive 7120.1 were not evaluated for use directly in ground product. If a company wants to include ground product in the intended use for their product, minimally FDA and FSIS would require studies supporting the request.

Please let me know if you have additional questions or issues you want to discuss.

Rachel Edelstein
Assistant Administrator
Office of Policy and Program Development
Food Safety and Inspection Service, USDA
202-550-4752 (Cell)
rachel.edelstein@usda.gov

Supporting_document_2

Correspondence from the FSIS New Technology Group/Office

From: FSIS New Technology
To: Frey, Sandra - FSIS
Subject: 7120.1 [Incident: 210216-000011]
Date: Tuesday, February 16, 2021 5:08:43 PM

Recently you requested personal assistance from our on-line support center. Below is a summary of your request and our response.

If this issue is not resolved to your satisfaction, you may reopen it within the next 14 days.

Thank you for allowing us to be of service to you.

To update this question by email, please reply to this message or to *access your question from our support site, click here*.

Subject

7120.1

Response By Email (JG@askFSIS) (02/16/2021 05:08 PM)

Hi Sandra,

Yes, the establishment may use SalmoFresh as indicated in the email.

I hope this helps.

Jennifer

askFSIS and Small Plant Help Desk are Moving to a New Platform

On February 19th, 2021 askFSIS and the Small Plant Help Desk will be transferred to a new data management platform. This change will result in some improvements. For example, in the new system, customers can simply submit their question directly from the web interface without the need to create an account or login. This change will also seamlessly connect askFSIS and the Small Plant Help Desk to Ask USDA and help further improve customer service. Existing customers will have until February 18th to save any of their submitted questions. You can find instructions for saving your questions on the askFSIS and Small Plant Help Desk account page.

Your message has been received by the Risk and Innovations Management Staff (RIMS) and is being assigned to a Staff Specialist for response.

Our goal is to provide an accurate response as quickly as possible—in most instances, this will be within two working days. However, Retained Water Protocol submissions will be answered within 30 days and New Technologies notification

and protocol submissions will be answered within 60 days.

If the response that you receive does not completely answer your technical concerns, you can telephone RIMS for additional discussion at 1-(301) 504-0884 between the hours of 7:00 a.m. and 4:00 p.m. ET, Monday through Friday. Please refer to the reference number when calling for clarification.

The reference number for your question is 210216-000011.

You may update your incident here.

Thank you
Risk and Innovations Management Staff

Customer By CSS Web (Sandra Frey) (02/16/2021 08:41 AM)

The establishment has been using the bacteriophage SalmoFresh for ground poultry for at least two years, as it was accepted for use in 2018. Recently the establishment was notified by labeling that the bacteriophage is not accepted for use on ground poultry. The bacteriophage manufacturer Intralytix reached out to FSIS concerning this rescindment, with FSIS discussing an error with labeling in understanding acceptable use for the bacteriophage (see SalmoFresh PDF attached)l.

The current 7120.1 restricts the bacteriophage use to prior to grinding (attached Dir. 7120 Nov 2019 page 24) whereas a 2018 version does not (attached 7120.1 v. 46 page 32).

Does the establishment need to wait for an update to FSIS Directive 7120.1 or the New Technology Table to return to using SalmoFresh on ground poultry? Or does the email support that bacteriophage use is OK for ground poultry?

From: Alexander Sulakvelidze
To: Hice, Stephanie
Subject: [EXTERNAL] RE: GRN 000966 - Questions for Notifier (USDA/FSIS)
Date: Thursday, May 6, 2021 2:37:11 PM
Attachments: image001.png
Response to FDA_additional information_May 6 2021.pdf

Dear Stephanie,

Attached our responses to the additional questions posed by the FSIS USDA regarding GRN 000966.

Please let me know if you have any further questions regarding the information provided, or if any of our answers requires further clarification.

Thank you!

Sandro Sulakvelidze

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Wednesday, April 28, 2021 1:25 PM
To: Alexander Sulakvelidze <asulakvelidze@intralytix.com>
Subject: RE: GRN 000966 - Questions for Notifier (USDA/FSIS)

Dear Dr. Sulakvelidze,

During review of GRAS Notice No. 000966, USDA/FSIS noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to USDA/FSIS's comments.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Theirs (what is this?)

Additional Information

In response to the FDA questions of April 28, 2021 regarding GRN #000966

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Questions/Comments Regarding GRN 000966 from USDA FSIS:

"The cocktail is between 3 to 8 phages, but the company only identified 3 of the phages. Is it possible they could explain the make-up of the mixture if more than the 3 identified phages are included? Will the identified 3 phages always be a part of the phage cocktail? Is there a reason the other 5 phages were not identified or included? Can they provide details on the additional 5 phages?"

There are three separate but related questions in this paragraph. We have addressed each of the questions individually below.

Is it possible they could explain the make-up of the mixture if more than the 3 identified phages are included?

The CampyShield™ preparation is envisioned as a cocktail of approximately equal concentrations of 3 to 8 lytic phages with lytic activity against *Campylobacter* spp. The rationale for using 3 to 8 phages to prepare a cocktail is to swiftly respond to new and emerging strains of *Campylobacter*, including multidrug-resistant or phage-resistant *Campylobacter* clones that may emerge in the future. Initially, the currently specified three phages will be included in the CampyShield™ preparation, however, if a new clone of *Campylobacter* emerges in the future that is not susceptible to the current three phage cocktail, additional phages lytic for *Campylobacter* will be added to increase the spectrum and/or potency, or the existing phages will be replaced with new phages to better target these newly emerged strains. In either scenario, the maximum number of phages in the cocktail will not exceed eight and the minimum number of phages will not be less than three.

Will the identified 3 phages always be a part of the phage cocktail?

For the foreseeable future, the same three phages will be present in the cocktail. In other words, we believe that the near-term cocktail updates will be adding new phages to the cocktail, rather than replacing the current three phages. But long-term, we may also completely refresh the cocktail with new lytic phages that are more potent against the *Campylobacter* strain populations predominant in food processing facilities at that time. In all instances, however, the number of

phages will not exceed 8 and will not be less than 3. Additional qualifying criteria are discussed below.

Is there a reason the other 5 phages were not identified or included? Can they provide details on the additional 5 phages?

We have not yet determined what additional 5 phages will be added to the CampyShield preparation over the next several years. For example, we may need to isolate new lytic phages with strong lytic potency against new *Campylobacter* strains that may emerge in food processing facilities in the United States one or two years from now. Therefore, it is currently not possible to specifically state which phages will be included in the cocktail in the future. However, we will ensure that (1) CampyShield™ preparation will always contain a minimum of 3 and maximum of 8 phages, (2) all new / substitute phages will meet the safety criteria set forth for the original three phages included in the current cocktail (including being strictly lytic and not containing any "undesirable" genes in their genomes, including functional portions of any of the toxin-encoding sequences described in 40 CFR 725.421(d)), (3) all new phages will be deposited with the American Type Culture Collection (ATCC), (4) all new / substitute phages will be manufactured using the same manufacturing protocols and will meet all of the same QC and release criteria (including purity and potency criteria) as those established for the current three phages in CampyShield, (5) CampyShield preparation will be blended using the same manufacturing protocols and will meet all of the same QC and release criteria (including lytic titer/potency, microbial purity, endotoxin, and identity criteria) as those established for the current version of CampyShield™ (the only difference being the number of phages in the preparation), and (6) irrespective of phage composition, all CampyShield preparations will be used as intended, up to the maximum application rate of 10^8 PFU/g of food, and as otherwise specified for the current three phage version described in this GRAS application.

Concluding remarks

Having the ability to rapidly update the CampyShield preparation as part of this GRN000966, to include 3 to 8 lytic phages with potency against *Campylobacter*, is an important factor for optimal phage biocontrol against *Campylobacter* under the intended conditions of use, and ultimately for

improving food safety by effectively reducing or eliminating *Campylobacter* contamination of foods with bacteriophages. Recognizing this need, the FDA has previously "approved" (i.e., did not have any questions) another phage cocktail that included 3 to 8 phages for use in various food safety applications (EcoShield PX™, GRN 834). The present GRAS notice is seeking similar regulatory status for CampyShield™ (GRN966), technically equivalent to the EcoShield PX™ (GRN 834) phage preparation which also contains 3 to 8 lytic bacteriophages not all of which have been identified by the time of GRN 834 GRAS status granting.

July 26, 2021

Questions/Comments Regarding GRN 000966 from USDA FSIS:

Comment

We noted an option to the submitter that they did not agree to and provided justification for.

We pointed out in our original response that the total amount of liquid added is $2.86/325\,x100 = 0.88\%$. The submitter is using GRN 435 as justification for listing accepting CampyShield for use in ground poultry as well. We went back to GRN 435, and the 0.88% used for CampyShield is much higher than the 0.04% used in GRN 435. As we indicated we are currently working with our leadership regarding our policy on adding phage directly to ground product. Considering this and the amount of liquid added, we still present the option of descriptive labeling for ground poultry under the understanding that the listing might be updated if their support aligns with the requirements of any future policy changes. The submitter may also opt to remove ground poultry until such a time that our policy is finalized and FSIS can re-evaluate.

If they decide to remove ground poultry, the listing would read that the intended use is for red meat including whole carcasses, primals, subprimals, cuts, trimmings, and organs and raw poultry including carcasses and parts.

From: Alexander Sulakvelidze
To: Hice, Stephanie
Subject: [EXTERNAL] RE: GRN 000966 - Questions for Notifier (USDA/FSIS)
Date: Tuesday, July 27, 2021 8:33:17 AM
Attachments: image001.png

Dear Stephanie,

In order to expedite processing of this GRAS application, we agree with the FSIS suggestion to remove ground poultry from the list. According to the FSIS, the resulting listing would read that the intended use is for red meat including whole carcasses, primals, subprimals, cuts, trimmings, and organs and raw poultry including carcasses and parts – presumably with no labeling requirements. Please go ahead and process the application as outlined above. We remain hopeful that FSIS will resolve the issue of adding phage directly to ground product in the not too distant future, at which point we hope to revisit this method of application.

Thank you!

Sandro

Alexander Sulakvelidze, Ph.D.
President and CEO
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www.intralytix.com

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Monday, July 26, 2021 2:29 PM
To: Alexander Sulakvelidze <asulakvelidze@intralytix.com>
Subject: RE: GRN 000966 - Questions for Notifier (USDA/FSIS)

Dear Dr. Sulakvelidze,

During review of GRAS Notice No. 000966, USDA/FSIS noted an additional comment that needs to be addressed and is attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to USDA/FSIS's comment.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Regulatory Review Scientist & Microbiology Reviewer
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

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