1. **Date:** October 27, 2010

2. **Name of submitter:** Alexander Sulakvelidze.

3. **Address:** Intralytix, Inc., 701 East Pratt St, Baltimore, Maryland 21202

4. **Description of the proposed action:**

The food contact substance is a product called EcoShield™, which is composed of three different strains of bacteriophages that have the ability to specifically and selectively kill the harmful *E. coli* strain O157:H7, which can cause a serious foodborne illness. The proposed use of the food additive is to act as an antimicrobial processing aid by reducing the level of surface contamination of red meat with *E. coli* O157:H7 prior to the meat grinding process. The product is (i) All natural (all component phages were isolated from the environment) and not genetically modified, (ii) Does not contain preservatives, (iii) Does not alter food flavor, aroma, or appearance, (iv) Does not contain any known, potentially allergenic substances, (v) Is certified both Kosher and Halal (with OMRI certification pending), and (vi) Is cost effective and cost competitive.

EcoShield™ is sold as a concentrate that is diluted (with water) 1:10; the use level concentration is applied to the parts and trim of the red meat at a rate of approximately 1 mL per 250 $cm^2$ of surface area.

Need for proposal

Foodborne illnesses are a substantial health burden in the United States. The Center for Disease Control estimates that each year, 76 million people get sick, 300,000 are hospitalized, and 5,000 die. In the U.S. alone, these illnesses are estimated to cause $37.1 billion annually in medical costs and lost productivity. According to the World Health Organization, foodborne illnesses result in 1.5 billion cases of diarrhea in children of which 300,000 lead to death.

Due to increased regulatory efforts and establishment of a zero-tolerance standard for *E. coli* O157:H7 ground beef and components of ground beef, processors have implemented more rigorous prevention and monitoring programs. These efforts led to a substantial decrease between the years 2001 and 2004. However, the trend for incidence of *E. coli* O157:H7 is again on the rise and close to reaching the original baseline. Thus, there is substantial need for new and effective anti-bacterial interventions. The risk of illness from *E. coli* O157:H7 is avoided by adequate cooking. However, in the case of ground beef, surface bacteria present at the time of grinding and mixing become incorporated throughout the finished product. Unless a hamburger made from the ground beef is cooked until the center is well done, a risk remains. The number of illnesses noted above is testament to the fact that meat is not always adequately cooked. Therefore, cooking should be regarded only as the last line of defense. The first line of defense should be to prepare ground meat from parts and trim of red meat that do not contain *E. coli* O157:H7. Thus, novel and effective approaches that will help remove or significantly reduce the level of surface contamination of red meat with *E. coli* O157:H7 are urgently required. Lytic bacteriophages provide one such approach.

Intended technical effect

EcoShield™ is intended to produce a statistically significant reduction of *E. coli* O157:H7 contamination compared to a water or carrier control when applied as directed to parts and trims of red meat. These and some additional efficacy studies performed with EcoShield™ (ECP-100) were reported in peer reviewed literature (Abuladze, Li et al. 2008).

Location

The food contact substance will be used in facilities that produce ground red meat.

Types of environment affected

Workplace at ground meat processing facilities

Disposal

Waters receiving liquid production wastes of any unused EcoShield™ left at the end of the workday will be disposed via municipal sewer

**5. Identification of substance that is the subject of the proposed action:**

Complete nomenclature:

The component phages in EcoShield™ were isolated by Intralytix's scientists from waters of the Chesapeake Bay / Inner Harbor water in Baltimore and from the commercial "Pyophage" preparation available for sale in the Republic of Georgia.

Phage Collection Date, as defined in the FCN:
ECML-4 Harbor water, Baltimore, MD 11/18/2004
ECML-117 Harbor water, Baltimore, MD 12/21/2004
ECML-134 Pyophage (Lot #052005) 3/8/2005

*Note:* The "Pyophage" preparation is a commercial phage-containing product for treating human skin infections. It is manufactured by the Eliava Institute of Bacteriophage in Tbilisi, Republic of Georgia (one of the former Soviet Union republics). All phages contained in Pyophage have been isolated from the environment.

The component phages in EcoShield™ are the members of the *Myoviridae* phage family, as defined by the International Committee on the Taxonomy of Viruses (ICTV) (http://www.ictvonline.org/) and by Ackermann and Berthiaume (Ackermann and Berthiaume 1995).

| Phage | Taxonomy | Head (nm) | Tail (nm) | Φ Length |
|---|---|---|---|---|
| ECML-4 | *Myoviridae* | 82 | 123 | 205 nm |
| ECML-117 | *Myoviridae* | 79 | 117 | 196 nm |
| ECML-134 | *Myoviridae* | 114 | 106 | 220 nm |

<u>CAS registration number</u>: Not applicable

<u>Molecular Weight/Formula:</u>

As the food contact substance is a bacteria phage and not a chemical, we have provided the genetic sequence to satisfy the molecular weight/formula request

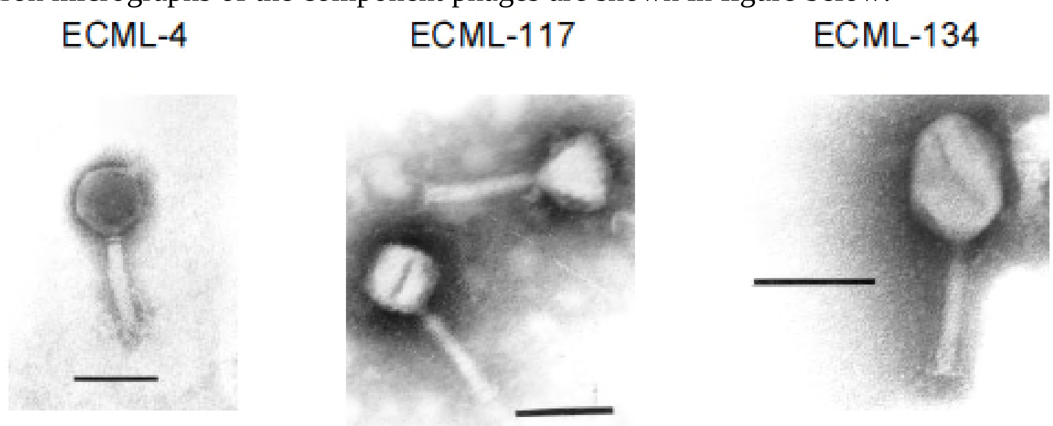
The three component monophages contained in EcoShield™ have been fully sequenced.

Full genome sequence of the three component monophages is enclosed:
Full genome sequence of ECML-4 Appendix 1
Full genome sequence of ECML-117 Appendix 2
Full genome sequence of ECML-134 Appendix 3

<u>Structural formula/physical description:</u>

As the food contact substance is a bacteria phage and not a chemical, we have provided the following morphological data to satisfy the structural formula/physical description request.

Electron micrographs of the component phages are shown in figure below:



Each of the electron micrographs contains a 0.1 µm calibration bar. Head and tail dimensions were obtained by direct measurement of the electron micrographs using a ruler, followed by normalization to the calibration bars to convert the measurements to nm. The dimensions of phages in EcoShield™ range from 79 to 114 nm in head length, and 106 to 123 nm in tail length.

**6. Introduction of substances into the environment:**

There are no extraordinary circumstances pertaining to the production of EcoShield™.

Factory-produced ground beef production occurs on a remarkably large scale. The entire factory-produced ground beef market in the United States is estimated to be 10 billion pounds. Intralytix estimates that given the challenges of market penetration and production limitations that it is unlikely to capture more than a small percentage of this market within the next 5 years. Please see attachment entitled "Confidential Environmental Information" for the percentage of market share, estimate of the maximum yearly market volume of food contact substance for the proposed use based on total fifth year production estimates. In one study (Pruett, Biela et al. 2002), the authors studied a typical production lot of ground beef patties of 53,960 kg produced over a single shift in a plant running at least two shifts. For the purposes of this assessment, we are assuming that 100% of the production is treated, that the factory runs two shifts, and that in round numbers, a large-scale producer will produce 108,000 kg of ground beef per day.

The amount of EcoShield™ that would be applied daily to ground beef can be calculated as follows:

- EcoShield™ application rate of 0.0028 mL per gram of beef trim or 2.8 mL per kg.

- Daily use of EcoShield™ would entail 2.8 mL/kg x 108,000 kg, or 302,400 mL, or 302 liters per day

- Yearly use of EcoShield™ would entail 302,400 mL, or 302 liters per day x 260 working days/year = 78,624,000 mL or 78,624 liters per year

*Note:* The calculation is based on the assumption that the daily processing amount of 108,000 kg were all processed (and treated with EcoShield™) at a single facility/locations. If multiple locations/facilities are used, the amount of EcoShield™ used per facility/location will be proportionally less.

Since EcoShield™ will be applied to the beef pieces immediately prior to grinding, we believe that virtually all applied EcoShield™ will be captured within the ground beef product. Small amounts (i.e., ca. 1%, according to industry standards for similar applications) of EcoShield™ that does not enter the grinder will be recovered by processing conveyors, and it typically will be sent to an in-house wastewater treatment system. Such systems routinely disinfect incoming wastewater with a halogen (bleach) solution. Because *E. coli* O157:H7-specific phages (and all other phages) are highly sensitive to halogen disinfectants, we expect that all of the EcoShield™ component phages that enter the in-house wastewater system will be inactivated. However, even if we assume that none of the phages is inactivated, we estimate that the worst-case amount of EcoShield™ that would contain viable phages and be available for discharge from a food-processing facility would be approximately:

302 L/day × 0.01 = 3 L/day.

Please note that the 3L/day estimate does not include any discharge of unused product at the end of the day. As mentioned above it is suggested that any used portions of EcoShield™ be disposed of down the drain at the end of the production day once the container of EcoShield™ has been opened. Although EcoShield™ will nominally be be sold in 5L containers, Intralytix will customize the container size to accommodate the production needs of individual clients. This will minimize the amount of unused EcoShield™ that will need to be discarded at each facility. We would not expect end of day disposal to exceed 0.5 L/day of the concentrate, which is approximately 10% of the 5L container. Therefore an estimate daily release including unused portions would be as follows:

3 L/day + 0.5L/day = 3.5 L/day

The component monophages of EcoShield™ may be continuously released into the environment via two main routes: (1) by passage through the GI tract of humans who consume EcoShield™-treated foods; and (2) from points of use (e.g., food-processing facilities). The possible environmental impact of both scenarios is discussed below. Only small amounts of phage will be released into the environment at the points of use (approximately 1% of total volume). The majority of phage specific for *E. coli* O157:H7 will be contained within the foods' packaging, where they will either be inactivated over time, or be inactivated through cooking and consumed by humans and be inactivated in the GI tract, particularly in the absence of host bacteria.

<u>Estimated wastewater effluents from meat processing facility</u>

The concentration of *E. coli* O157:H7-specific phages in the EcoShield™ "working concentration" solution is approximately $10^9$ PFU/mL after a 1:10 dilution with water with the article of commerce. The article of commerce, which includes all three strains of phages, has a concentration of $10^{10}$ pfu/mL. The fate of *E. coli* O157:H7-specific phages in wastewater/sewage may be estimated using data available for other phages. These data would suggest that the component monophages of EcoShield™ are not likely to multiply in sewage. For example, coliphages and other phages have been reported (Havelaar, Pot-Hogeboom et al. 1990; Lasobras, Dellunde et al. 1999; Leclerc, Edberg et al. 2000) to be unable to multiply in sewage and in the environment in general. Nevertheless, for the purpose of this assessment, we assume that all monophages will multiply 10-fold in the environment, which means that our working solution concentration of $10^9$ pfu/mL is estimated to become $10^{10}$ pfu/mL, which is the concentrations we have used for the following calculations. Therefore, the maximum phage released in the environment is estimated as:

> Daily release of phages into the environment would be 3.5 liters of 1 x $10^{10}$ PFU/mL per day = 3.5 x $10^{13}$ PFU/day
>
> Yearly release of phages into the environment would be (3.5 x $10^{13}$ PFU/day) x (260 working days/year) = 9.1 x $10^{15}$ PFU/year

The environmental release calculated above for ground beef represents a maximal figure.

<u>Estimated environmental release by passage through the GI tract of humans (solid waste)</u>

After applying EcoShield™ onto foods, it is likely that the levels of the preparation's component monophages will decrease over time and they eventually may be completely eliminated from the treated foods (because the component monophages of EcoShield™ can not multiply and increase in number on foods in the absence of their host *E. coli* O157:H7 strains). If EcoShield™-treated foods are heat treated, all of its component monophages are likely to be inactivated under the same conditions as the comparable *Listeria monocytogenes*-specifc phage in a related, FDA-approved product, LMP-102 (LMP-102 phages are inactivated under conditions that mimic cooking for ≥ 2 min, or by microwaving for ≥ 1 min; FAP 2A4738). In addition, even if EcoShield™-treated foods are uncooked before consumption, most, if not all, of the component monophages are likely to be inactivated by the low pH of gastric secretions unless encapsulated in a host *E. coli* O157:H7. Thus, we believe that environmental release of the phages contained in EcoShield™, by passage through the GI tract of humans who consume EcoShield™-treated foods, will be insignificant. Moreover, such release will not be localized to one or a few food processing facilities, but will be spread geographically to all locations where EcoShield™-treated foods have been distributed or consumed. The estimated environmental concentrations resulting from EcoShield™ use in food–processing facilities are discussed below.

<u>Estimated environmental concentrations from EcoShield™ use</u>

EcoShield™ waste solution containing viable *E. coli* O157:H7-specific phages will be discharged to either a point source, e.g. pond, lake, river or to a Publicly-Owned Treatment Works (POTW). Environmental concentrations of *E. coli* O157:H7-specific phages, and the possible environmental impact for both types of discharges, are presented below.

<u>Point-source discharge</u>
It is assumed that a worst-case point-source discharge will be to a small circular lake or pond. For the purposes of this assessment, we have assumed that the pond has a diameter of 100 meters and an average depth of two meters.

The pond volume is then:

$\pi r^2 \times$ depth = $\pi \times 50^2 \times 2$ = 15,708 $m^3$= 1.6 x $10^7$ L

and the *E. coli* O157:H7-specific phage concentration is then:

(3 L ÷ 1.6 x $10^7$ L) × (1 x $10^{10}$ PFU/mL) = 1.88 x $10^3$PFU/mL

Although it is unlikely that a large food-producing facility will use a small circular lake or pond having a diameter of 100 meters as its point-source discharge (as much larger reservoirs are likely to be used for that purpose), even for such small reservoir the concentrations of phages are negligible. In order to survive and multiply, phage specific for *E. coli* O157:H7 must co-exist with their host bacterium. Since it is highly unlikely that such reservoirs would harbor large number (or any) *E. coli* O157:H7, it is anticipated that the bacteriophage would become undetectable over a relatively brief period of time. Figures do exist for the levels of *E. coli* O157:H7 bacteriophage in sewage (Muniesa and Jofre 2004), where the bacterial host can be present at low levels. Typical phage concentrations for all phage infecting *E. coli* O157:H7 ranged from 5.1 x $10^2$/mL to 5.5 x $10^2$/mL.

<u>POTW discharge</u>

The concentration of *E. coli* O157:H7-specific phages entering a POTW can be calculated by estimating the amount of water inflow into a POTW and the percentage of inflow into a POTW flow from a food-processing plant. According to a 1995 USGS report (Solley, Pierce et al. 1995), the per capita domestic use of water is approximately 100 gallons per day. Assuming a small city with a population of 50,000, the total daily inflow into the POTW is:

50,000 × 100 gal/person/day = 5 x $10^6$gal/day or 2 x $10^7$L/day

Regarding the percentage of POTW inflow that comes from a food processing plant, data from a 1986 study of POTWs (Wetzel and Murphy, 1986;) indicated that commercial use inflow is approximately 25%. While the percentage use of a single industrial facility is difficult to estimate, we believe that 50% is standard for a high user. Therefore, the estimate of flow percentage that is contributed for a food-processing facility is:

25% × 50% = 12.5%

and the concentration of *E. coli* O157:H7-specific phages in the POTW influent is then:

3 L/day ÷ 2 x $10^7$L/day × 12.5% × 1 x$10^{10}$PFU/mL = 1.9x $10^2$ PFU/mL

As above, these concentrations of phages are low, and would be expected to approach zero rapidly in the absence of the *E. coli* O157:H7 host. The POTW phage concentrations are approximately 30% of those in sewage, 5.1 x $10^2$/mL – 5.5 x $10^2$/mL (Muniesa and Jofre 2004) of *E. coli* O157:H7-specific bacteriophage in sewage, and to about 1.8 x $10^{-22}$ - 1.8 x $10^{-24}$ % of the estimated total phage population of $10^{30}$- $10^{32}$ PFU, respectively, on Earth.

Please see Appendix 4 for the MSDS.

**7) Fate of Emitted Substances in the Environment**

Data support the notion that phage specific for *E. coli* O157:H7 would disappear from the environment, particularly in the absence of host bacteria. Although rigorously obtained data pertaining to the kinetics of *E. coli* O157:H7 phage inactivation in the environment are not available, several studies have determined the survivability of other phages in the environment (including sewage), and the effect of various environmental conditions on their inactivation rates (Lasobras, Dellunde et al. 1999; Sinton, Finlay et al. 1999). In general, the inactivation rates vary among various phages, and they are dependent on various environmental conditions such as temperature, solar radiation, etc. In laboratory experiments, Lasobras et al. (Lasobras, Dellunde et al. 1999) found that the levels of coliphages and *B. fragilis*-specific phages in sewage samples declined by about 30% in 10 days (the first sampling time); whereas, the concentration of F specific bacteriophages declined by > 90% during the same time period. The same authors reported that the inactivation rates of *B. fragilis* phages and somatic coliphages in sewage were the lowest among all of the phages (including the F-specific phages) examined. Thus, *E. coli* O157:H7-specific phages may be inactivated faster than *B. fragilis* phages and somatic coliphages in the environment. Also, in the above-referenced study, the survivability of phages was determined under somewhat artificial conditions; i.e., phage-containing samples were protected from sunlight and other environmental factors likely to have a significant negative impact on phage viability. Indeed, much faster phage inactivation rates (i.e., inactivation rates of ca. 95% and 99.9% – in two days – for somatic coliphages and F-RNA phages, respectively) were reported by Sinton et al. (Sinton, Finlay et al. 1999), who analyzed phage stability in sewage-polluted seawater under conditions mimicking natural exposure to sunlight and other environmental factors. In conclusion, given the dilute concentration of phages released into the sewage system and the unfavorable environmental conditions found in a sewage system, it is likely that all or at least a significant percentage of phages in EcoShield™ will become inactivated.

Below is the Composition of undiluted EcoShield™

| Components of undiluted EcoShield™ | |
|---|---|
| **Component** | **Concentration (ppm)** |
| Component phages | 0.24 |
| Total Organic Carbon | up to 500 |
| Sodium | 3,400–3,900 |
| Potassium | 150–225 |
| Chloride | 5000–5,399 |
| Water | ~ 990,000 |

<u>Previous regulatory clearances for EcoShield™ components</u>

Aside from the phage components, most starting components of EcoShield™ have an existing regulatory status as regulated GRAS ingredients or additives.

*Peptones:* Peptones are GRAS affirmed at 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. They are not a component of the animal-product free growth media (NZCYM broth) used in EcoShield™ production.

*Yeast Extract:* Yeast extract is a commonly used food ingredient. For example, baker's yeast extract is GRAS affirmed as a flavoring agent or adjuvant at up to 5% in foods generally. 21 CFR § 184.1983. Since the total organic carbon TOC) specification for EcoShield™ is ≤ 50mg/L, any remaining yeast extract is present at safe levels.

*Salts.* Salts present in EcoShield™ are primarily from phosphate-buffered saline (PBS); other salts in EcoShield™ are in generally low levels.

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

*Potassium Phosphate, dibasic:* Potassium phosphate, dibasic, "dipotassium phosphate" is GRAS listed as a sequestrant under 21 CFR § 182.6285. A proposed regulation would have affirmed dibasic potassium phosphate as GRAS for additional uses, including as a pH control agent (the reason it is used in EcoShield TM) at levels not exceeding good manufacturing practice. 44 Fed. Reg. at 74855 (December 18, 1979). Like many other GRAS affirmation proposals that were not finalized, this one was withdrawn on April 22, 2003 because FDA wanted "to reduce its regulatory backlog and focus its resources on current public health issues," and not because of any concerns about its safety. 68 Fed. Reg. 19766. Petitioner is not aware of any new data that would call into question the GRAS status of this compound for its intended use.

*Potassium Phosphate, monobasic:* Potassium phosphate, monobasic, was also the subject of a proposed regulation, which would have affirmed it as GRAS for use as a pH control agent (the reason it is used in EcoShield™), among other uses, at levels not to exceed good manufacturing practice (see discussion above under "Potassium Phosphate, dibasic"). Petitioner is not aware of any new data that would call into question the GRAS status of this compound for its intended use.

*Glycerol:* Glycerol "glycerin" is GRAS listed under 21 CFR § 182.1320 as a multiple purpose food substance when used in accordance with good manufacturing practice.

*DNase:* DNase is sourced from bovine pancreas. As a protein, DNase is a polypeptide that would contribute to detectable levels of amino acids following hydrolysis.

*RNase:* RNase is sourced from bovine pancreas. As a protein, RNase is a polypeptide that would contribute to detectable levels of amino acids following hydrolysis.

*Antifoaming agent:* P2000 antifoam is polypropylene glycol-based, Kosher-certified product, approved for a variety of food additive uses, both direct and indirect (The Dow Chemical Company, Midland, Michigan; http://www.dow.com)

The following list summarizes support for the inherent safety of EcoShield™:

Maximum daily exposure to phages from EcoShield™ is 0.0315 ppb in the diet.

Total organic carbon (TOC) is ≤ 50 ppm in EcoShield™ and represents safe components on the basis of a history of prior human exposure

Phages are safe in general, based of a history of prior human exposure and their ubiquity in the environment and the lack of any genes associated with toxicity.

Below is a list of undesirable genes screened for in EcoShield™ and found to not be present.

| Sequence source | Toxin name |
|---|---|
| *Corynebacterium diphtheriae & C. ulcerans* | Diphtheria toxin* |
| *Pseudomonas aeruginosa* | Exotoxin A; Proteases; Leucocidin (leukocidin, cytotoxin*) |
| *Shigella dysenteriae* | Shigella toxin* (Shiga toxin, *Shigella dysenteriae* type toxin, Vero cell toxin) |
| *Clostridium botulinum* | Neurotoxins A, B, C1*, D, E, F, G (Botulinum toxins, botulinal toxins); Lysin; C2 toxin |
| *Clostridium tetani* | Tetanus toxin (tetanospasmin); Tetanolysin |
| *Proteus mirabilis* | Neurotoxin |
| *Staphylococcus aureus* | Alpha toxin (alpha lysin); Beta-lysin (beta toxin); Gamma lysin (Gamma toxin); Enterotoxins (SEA*, SEB, SEC, SED SEE); Pyrogenic exotoxins A B; Toxic shock syndrome toxins (TSST-1); Leucocidin (leukocidin, cytotoxin) |
| *Yersinia pestis* | Murine toxin |
| *Bacillus alve* | Alveolysin |
| *Bacillus cereus* | Cereolysin; Enterotoxin (diarrheagenic toxin, mouse lethal factor) |
| *Bacillus laterosporus* | Laterosporolysin |
| *Bacillus thuringiensis* | Thuringiolysin |
| *Clostridium bifermentans* | Lysin; Lecithinase |
| *Clostridium caproicum* | Lysin |
| *Clostridium chauvoei* | Delta-toxin |
| *Clostridium histolyticum* | Epsilon-toxin |
| *Clostridium novyi* | Gamma-toxin |
| *Clostridium oedematiens* | Delta-toxin |

| Sequence source | Toxin name |
|---|---|
| *Clostridium perfringens* | Theta-toxin (Perfringolysin); Alpha-toxin (phospholipase C, lecithinase); Enterotoxin; Beta-toxin; Delta-toxin; Epsilon-toxin; Kappa-toxin |
| *Clostridium septicum* | Delta-toxin |
| *Clostridium sordellii* | Lysin |
| *Listeria monocytogenes* | Listeriolysin (A B) |
| *Streptococcus pneumoniae* | Pneumolysin |
| *Bacillus anthracis* | Edema factor (Factors I II); Lethal factor (Factors II III) |
| *Bordetella pertussis* | Adenylate cyclase (Heat-labile factor); Pertussigen (pertussis toxin, islet activating factor, histamine sensitizing factor, lymphocytosis promoting factor) |
| *Clostridium difficile* | Enterotoxin (toxin A); Cytotoxin (toxin B) |
| *Escherichia coli* & other Enterobacteriaceae spp. | Heat-labile enterotoxins (LT); Heat-stable enterotoxins (STa, ST1 subtypes ST1a, ST1b; also STb, STII); Cytotoxin (Shiga-like toxin, Vero cell toxin) |
| *Legionella pneumophila* | Cytolysin |
| *Vibrio cholerae* & *Vibrio mimicus* | Cholera toxin* (choleragen) |
| Other *Clostridium* spp. | Lecithinase |
| *Corynebacterium pyogenes* & other *Corynebacterium* spp. | Cytolysin (phospholipase C), Ovis toxin (sphingomyelinase D) |
| *Aeromonas hydrophila* | Aerolysin (beta-lysin, cytotoxic lysin) |
| *Streptococcus pyogenes* | Streptolysin S (SLS); Erythrogenic toxins* (scarlet fever toxins, pyrogenic exotoxins) |
| *Yersinia enterocolitica* | Heat-stable enterotoxins (ST) |

In addition to the above-listed genes, Intralytix screenecd the phage genomes for the presence of all other known toxin genes and bacterial 16S RNA genes. None were found. Therefore there is no risk of the transfer of undesirable genes from EcoShield™.

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

The typical safety evaluation for a new food additive submitted to the FDA relies heavily on toxicology studies in animals on the primary chemical ingredient. In the present case, the primary active ingredient is not a single chemical substance but a mixture of naturally occurring bacteriophages. The only other compounds are low levels of production byproducts, total organic solids (TOS), and some common salts. For these reasons the typical safety evaluation format is not applicable. Note that since EcoShield™ is applied directly to food, the analyses in this section assume 100% migration to treated foodstuffs

| | |
|---|---|
| **Physical/chemical characterization** | |
| Water Solubility | N/A |
| Dissociation Constant(s) | N/A |
| Octanol/Water Partition Coefficient (Log $K_{ow}$) | N/A |
| Vapor Pressure or Henry's Law Constant | N/A |
| **Depletion mechanisms** | |
| Sorption/Desorption ($K_{oc}$) | N/A |
| Hydrolysis | N/A |
| Aerobic Biodegradation | Estimated 90-95% within 48 hours |
| Soil Biodegradation | Estimated 90-95% within 48 hours |
| Photolysis | Reduced growth in presence of light |
| Metabolism | N/A |
| **Environmental effects**[1] | |
| Microbial Inhibition | specific pathogen for *E. coli* O157:H7 |
| Acute Toxicity | N/A |
| Chronic Toxicity | N/A |

## 8) Environmental effects of released substances:

The component monophages of EcoShield™ are naturally occurring, and they were all isolated from the environment. Two of the three phages (ECML4 and ECML117) were originally isolated in the United States, and therefore are already found in the environment. The third phage, ECML-134, although isolated in the Republic of Georgia, cannot necessarily be considered to be endemic to the region. There may be specific and special circumstances in which certain strains of phage may be conditionally called "endemic" to a specific region. Isolated areas or extremely unique and isolated environments, such as stromatolites, would provide situations in which progeny of one phage may predominate, creating an "endemic phage" in that isolated environmental niche. However, the conditions in the Georgian water estuary where ECML-134 was initially isolated cannot be regarded as isolated or unique. In general, most phages due to their rapid replication cycle, vast numbers, recalcitrance, and ability to be transported do not exhibit biogeographical patterns. In other words, phages isolated in various countries around the world are fairly similar within their taxonomical grouping (i.e., there are no "American" or "European" *Myoviridae* phages).

ECML-134 is a component of a bacteriophage-product marketed under the brand name Pyophage (sometimes transliterated as Piophage) in Georgia, and is an over-the-counter medication. It has been used in Georgia as a topical antimicrobial treatment for many years. Several articles describing the use of Pyophage have been published in English scientific literature (Markoishvili, Tsitlanadze et al. 2002; Jikia, Chkhaidze et al. 2005) and were also highlighted in the journal Science (Stone 2002). The Phage Therapy Center (http://www.phagetherapycenter.com/pii/PatientServlet?command=static_home&secnavpos=-1&language=0) in Georgia treats patients from various countries (including the United States) using Pyophage and other locally isolated bacteriophages. Various media articles have reported stories about Canadian and US residents traveling to Georgia to receive treatment with locally isolated bacteriophages. While there is no easy way to document the extent to which travelers from Georgia (including tourists and patients from the US returning from Georgia, and Georgian residents visiting the US) may have brought the product to the U.S., but it is unreasonable to assume it has not occurred. Performing a screen to try to isolate ECML-134 in the U.S. would be almost impossible as the phage most likely has evolved since it was first collected, and the geographic distribution of a majority of phage strains are transient. We have evidence from analyzing water samples from the National Harbor in Baltimore that the two other phages in EcoShield™ initially isolated in those waters were not found again in at least 3 subsequent samplings.

As noted above, phages isolated in various countries around the world are fairly similar, and ECML-134 is not an exception. Genomic analysis of the ECML-134 has shown high homology to domestic phage strains in the U.S. It also belongs to the same family of *Myoviridae* phages as the other two component phages included in EcoShield™. In addition, the following further illustrate the inability of ECML-134 to have sustainable growth once released into the environment:

1) 90-95% of phages disappear in a period of two days without the presence of the appropriate host (Sinton, Finlay et al. 1999).
2) Bacteria hosts and bacteria phages have successfully been co-evolving for billions of years, and it is extremely unlikely that the intended use of ECML-134 would alter this relationship to an appreciable degree.
3) Like all lytic phages in general, ECML-134 only targets a narrow range of *E. coli* species and is not broad-spectrum for *E. coli* or other organisms.

Accordingly, there are three reasons that the proposed use of EcoShield™ will not have an appreciable impact on the overall environmental levels of *E. coli* O157:H7-specific phages. First, very small amounts of *E. coli* O157:H7-specific phages (compared to the estimated levels of *E. coli* -specific and other phages naturally present in the environment) will be introduced into the environment, even under the most aggressive theoretical use scenario for EcoShield™. Secondly, only small amounts of phage will be released into the environment at the points of use. The majority will be contained within the treated food and its packaging, where they will either be inactivated over time, or by end user food preparation (cooking), or be consumed by humans and be inactivated in the GI tract. Finally, the small numbers of released phages are not likely to multiply in the environment, but, instead, are likely to be rapidly inactivated.

In conclusion, the dilute and small amount of ECML-134 released into the environment as the result of EcoShield™ use is truly negligibly even under the absolute maximal use scenario. Therefore, there are extremely low chances for ECML-134 to encounter hosts in the environment because of insufficient densities of either the phage or the host for both inhibit sustainable growth.

Bacteriophages are the most ubiquitous organisms on earth. For example, one milliliter of non-polluted stream water has been reported (Bergh, Borsheim et al. 1989) to contain approximately 2 x $10^8$ PFU of phages/mL, and the total number of phages on this planet has been estimated to be in the range of $10^{30}$– $10^{32}$ (see http://www.phage.org/index.htmL and Brussow and Hendrix 2002). This abundance of phages in the environment, and the continuous exposure of humans to them, explains the extremely good tolerance of the human organism to phages.

Phages also have been administered to humans via various sera and FDA approved vaccines commercially available in the United States (Merril, Friedman et al. 1972; Milch and Fornosi 1975; Moody, Trousdale et al. 1975). The biology of phages has been exhaustively studied. These studies have clearly shown that phages are obligate intracellular parasites of bacteria and are not infectious in humans or other mammals.

Bacteriophages are common commensals of the human gut, and they are likely to play an important role in regulating the diversity and population structure of various bacteria in human GI tracts. Phages capable of infecting *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 (Furuse, Osawa et al. 1983; Havelaar, Furuse et al. 1986; Calci, Burkhardt et al. 1998). 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 (Breitbart, Hewson et al. 2003).

No adverse immunologic or allergic sequelae have ever been reported because of human or animal exposure to phages (Alisky, Iczkowski et al. 1998; Sulakvelidze, Alavidze et al. 2001).

Bacteriophages are commonly consumed by humans via drinking water (Grabow and Coubrough 1986; Armon and Kott 1993; Lucena, Muniesa et al. 1995; Armon, Araujo et al. 1997).

Bacteriophages are commonly consumed by humans via various foods. In this context, bacteriophages have been commonly 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 (Whitman and Marshall 1971; Kennedy, Oblinger et al. 1984; Kennedy, Wei et al. 1986; Gautier, Rouault et al. 1995; Hsu, Shieh et al. 2002; Atterbury, Connerton et al. 2003; Greer 2005). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophages. To give just a few examples, bacteriophages were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed by Kennedy et al. (Kennedy, Oblinger et al. 1984). The levels ranged from 3.3 to 4.4 x $10^{10}$ PFU/100 g of fresh chicken, up to 3.5 x $10^{10}$ PFU/100 g of fresh pork, and up to 2.7 x $10^{10}$ PFU/100 g of roast turkey breast samples. In another study (Kennedy, Wei et al. 1986), samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined.

Because of the highly specific nature of their lytic cycle, and because of the extremely common exposure of humans to bacteriophages (including daily consumption of bacteriophages with various foods and drinking water), bacteriophages do not deleteriously affect the GI microflora. For example, when *E. coli*-specific phage T4 was administered orally to 15 healthy adult volunteers, it did not cause a decrease in total fecal *E. coli* counts. In addition, 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 (Bruttin and Brussow 2005).

Bacteriophages are commonly consumed by animals (including agriculturally important species) via various foods. For example, in a recent study from Texas A&M University (Maciorowski, Pillai et al. 2001), 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.

Based on information present in Format Items 7, phage specific for *E. coli* O157:H7 will disappear from the environment in the absence of host bacteria. Therefore EcoShield™ will not persist in the environment and not have the potential to bioaccummulate nor be continuously introduced into the environment at significant levels.

Based on environment fate and effects information provided under Format Items 7 and 8, the proposed use of EcoShield™does not present unique emissions circumstance that would threaten a violation of such laws and regulations.

**9) Use of resources and energy:**

Good food manufacturing practices may involve a number of steps to prevent and control bacterial contamination. As noted in Section 4(b), current control techniques are not completely effective. Since the efficacy of EcoShield™ is so narrowly focused, it is likely to supplement rather than replace existing microbial control practices. The product itself is more than 99.9% water. The dilution water required at point of use is a small fraction of total water consumption in a meat processing facility for general sanitation purposes. The impact on resources and energy will be trivial.

**10) Mitigation measures:**

There are no mitigation measures required for the food contact substance. Based on a review of adequate and complete data and information, no adverse environmental effects have been identified.

**11) Alternatives to the proposed action:**

There are no potential adverse environmental impacts identified with the production or use of EcoShield™, therefore there is no need to identify reasonable alternatives to the proposed action. An alternative is to not clear the food contact substance and continue to accept the current toll on public health and resources caused by *E. coli* O157:H7 contamination of ground meat.

**12) List of preparers:**

EA preparation performed by**:**

1) Alexander Sulakvelidze, Ph.D.
   Chief Scientist / Phage biologist
   Intralytix, Inc. 1)

2) Alice Chen, Ph.D.
   Staff Scientist / Molecular Biologist
   Keller and Heckman LLP

EA preparation preformed in consultation with:

John Dubeck
Partner
Keller and Heckman LLP

David Ettinger
Partner
Keller and Heckman LLP

Xin Tao
Legal intern
Keller and Heckman LLP

13) **Certification:**

"The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of Intralytix, Inc."

October 27, 2010

[redacted]

John Dubeck
Counsel for Intralytix, Inc.

14) **References:**


Abuladze, T., M. Li, et al. (2008). "Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by *Escherichia coli* O157:H7." Appl Environ Microbiol **74**(20): 6230-6238.

Ackermann, H. W. and L. Berthiaume (1995). Atlas of virus diagrams. Boca Raton, FL., CRC Press.

Alisky, J., K. Iczkowski, et al. (1998). "Bacteriophages show promise as antimicrobial agents." J Infect **36**(1): 5-15.

Armon, R., R. Araujo, et al. (1997). "Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries." J Appl Microbiol **83**(5): 627-633.

Armon, R. and Y. Kott (1993). "A simple, rapid and sensitive presence/absence detection test for bacteriophage in drinking water." J Appl Bacteriol **74**(4): 490-496.

Atterbury, R. J., P. L. Connerton, et al. (2003). "Isolation and characterization of *Campylobacter bacteriophages* from retail poultry." Appl Environ Microbiol **69**(8): 4511-4518.

Bergh, O., K. Y. Borsheim, et al. (1989). "High abundance of viruses found in aquatic environments." Nature **340**(6233): 467-468.

Breitbart, M., I. Hewson, et al. (2003). "Metagenomic analyses of an uncultured viral community from human feces." J Bacteriol **185**(20): 6220-6223.

Brussow, H. and R. W. Hendrix (2002). "Phage genomics: small is beautiful." Cell **108(**1): 13-6.

Bruttin, A. and H. Brussow (2005). "Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy." Antimicrob Agents Chemother **49**(7): 2874-2878.

Calci, K. R., W. Burkhardt, 3rd, et al. (1998). "Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters." Appl Environ Microbiol **64**(12): 5027-5029.

Furuse, K., S. Osawa, et al. (1983). "Bacteriophage distribution in human faeces: continuous survey of healthy subjects and patients with internal and leukaemic diseases." J Gen Virol **64 (Pt 9)**: 2039-2043.

Gautier, M., A. Rouault, et al. (1995). "Occurrence of *Propionibacterium freudenreichii* bacteriophages in swiss cheese." Appl Environ Microbiol **61**(7): 2572-2576.

Grabow, W. O. and P. Coubrough (1986). "Practical direct plaque assay for coliphages in 100-mL samples of drinking water." Appl Environ Microbiol **52**(3): 430-433.

Greer, G. G. (2005). "Bacteriophage control of foodborne bacteria." J Food Prot **68(**5): 1102-1111.

Havelaar, A. H., K. Furuse, et al. (1986). "Bacteriophages and indicator bacteria in human and animal faeces." J Appl Bacteriol **60**(3): 255-262.

Havelaar, A. H., W. M. Pot-Hogeboom, et al. (1990). "F-specific RNA bacteriophages and sensitive host strains in faeces and wastewater of human and animal origin." J Appl Bacteriol **69**(1): 30-7.

Hsu, F. C., Y. S. Shieh, et al. (2002). "Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat." J Food Prot **65**(1): 93-99.

Jikia, D., N. Chkhaidze, et al. (2005). "The use of a novel biodegradable preparation capable of the sustained release of bacteriophages and ciprofloxacin, in the complex treatment of multidrug-resistant Staphylococcus aureus-infected local radiation injuries caused by exposure to Sr90." Clin Exp Dermatol 30(1): 23-26.

Kennedy, J. E., Jr., C. I. Wei, et al. (1986). "Methodology for enumeration of coliphages in foods." Appl Environ Microbiol **51**(5): 956-962.

Kennedy, J. E. J. and J. L. Oblinger (1984). "Recovery of coliphages from chicken, pork sasuage, and delcatessen meats." Journal of Food Protection **47**(8): 623-626.

Lasobras, J., J. Dellunde, et al. (1999). "Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges." J Appl Microbiol **86**(4): 723-729.

Leclerc, H., S. Edberg, et al. (2000). "Bacteriophages as indicators of enteric viruses and public health risk in groundwaters." J Appl Microbiol **88**(1): 5-21.

Lucena, F., M. Muniesa, et al. (1995). "Simple concentration method for bacteriophages of *Bacteroides fragilis* in drinking water." J Virol Methods **54**(2-3): 121-30.

Maciorowski, K. G., S. D. Pillai, et al. (2001). "Presence of bacteriophages in animal feed as indicators of fecal contamination." J Environ Sci Health B **36**(5): 699-708.

Markoishvili, K., G. Tsitlanadze, et al. (2002). "A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds." Int J Dermatol 41(7): 453-8.

Merril, C. R., T. B. Friedman, et al. (1972). "Isolation of bacteriophages from commercial sera." In Vitro **8**(2): 91-93.

Milch, H. and F. Fornosi (1975). "Bacteriophage contamination in live poliovirus vaccine." J Biol Stand **3**(3): 307-310.

Moody, E. E., M. D. Trousdale, et al. (1975). "Bacteriophages and endotoxin in licensed live-virus vaccines." J Infect Dis **131**(5): 588-591.

Muniesa, M. and J. Jofre (2004). "Abundance in sewage of bacteriophages infecting *Escherichia coli* O157:H7." Methods Mol Biol **268**: 79-88.

Pruett, W. P., Jr., T. Biela, et al. (2002). "Incidence of *Escherichia coli* O157:H7 in frozen beef patties produced over an 8-hour shiftt." J Food Prot **65**(9): 1363-1370.

Sinton, L. W., R. K. Finlay, et al. (1999). "Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater." Appl Environ Microbiol **65**(8): 3605-3613.

Solley, W. B., R. R. Pierce, et al. (1995). Estimated use of water in the United States in 1995. U.S. Geological Survey Circular 1200, U.S. Geological Survey.

Stone, Richard (2002). "Stalin’s Forgotten Cure." Science **298**(5594): 728-31.

Sulakvelidze, A., Z. Alavidze, et al. (2001). "Bacteriophage therapy." Antimicrob Agents Chemother **45**(3): 649-659.

Summers, W. C. (2001). "Bacteriophage therapy." Annu Rev Microbiol **55**: 437-451.

Wetzel, E.D. ; Murphy, S.B. (1987) "Interferences at Publicly Owned Treatment Works." A technical report prepared by the Montgomery Consulting Engineer, Inc. for the Environmental Protection Agency. Report number PB-90-108853/XAB

Whitman, P. A. and R. T. Marshall (1971). "Isolation of psychrophilic bacteriophagehost systems from refrigerated food products." Appl Microbiol **22**(2): 220-223.

# *EcoShield*™ (ECP-100™) *Escherichia coli* Specific Phage Preparation

## Section 1. Product and Company Identification

| | |
|---|---|
| Product Name: | EcoShield™<br>(ECP-100™)<br>(Phage preparation effective against *Escherichia coli*) |
| Catalog Number: | ECP100 |
| Manufacturer: | Intralytix, Inc.<br>Columbus Center<br>701 E. Pratt St.<br>Baltimore, MD 21202 |
| For More Information Call: | 1-877-ITX-PHAGE<br>Monday – Friday 9:00 AM – 5:00 PM |

## Section 2. Composition and Information on Ingredients

| | |
|---|---|
| Component % by weight | *Escherichia coli* – specific phages < 0.00001<br>Phosphate Buffered Saline > 99.9 |
| CAS Number: | Not applicable. |

## Section 3. Hazards Identification

| | |
|---|---|
| Physical State and Appearance: | Clear, colorless to pale-amber liquid. |
| Emergency Overview: | |
| Potential Health Effects from: | |
| Inhalation: | Inhalation is unlikely to cause injury, excessive amounts of vapor or mist may cause mild irritation in the nose and/or throat. |
| Skin Contact: | Contact is unlikely to cause injury, but may cause mild redness or irritation. |
| Eye Contact: | Contact is unlikely to cause injury, but may cause mild redness or irritation. |
| Ingestion: | Ingestion is unlikely to cause injury, but may cause mild stomach ache or distress. |
| Routes of Entry: | Ingestion. Inhalation. |

## Section 4. First Aid Measures

| | |
|---|---|
| Eye Contact: | Flush with plenty of water. Get medical attention if irritation develops or persists. |
| Skin Contact: | Wash with soap and water. Remove contaminated clothes and wash before reusing. Seek medical attention if irritation develops or persists. |
| Inhalation: | If exposed to excessive levels of vapors or mists, remove to fresh air and get medical attention if cough or other symptoms develop. |
| Ingestion: | If swallowed, wash out mouth with water. Seek medical attention if symptoms appear. |

## Section 5. Fire Fighting Measures

| | |
|---|---|
| Flammability of the Product: | Non-flammable. |
| Auto-ignition: | Not applicable. |
| Flash Points: | Not applicable. |
| Flammable Limits: | Not applicable. |
| Fire Fighting Instructions: | Not applicable. |
| Protective Clothing (Fire): | Not applicable. |
| Special Remarks on Fire Hazards: | Not applicable. |
| Special Remarks on Explosion Hazards: | Not applicable. |

## Section 6. Accidental Release Measures

| | |
|---|---|
| Small Spill and Leak: | Mop up or absorb with an inert dry material and place in an appropriate waste disposal container. |
| Large Spill and Leak: | Absorb with an inert material and put the spilled material in an appropriate waste disposal container. |
| Spill Kit Information: | No specific spill kit is required for this product. |

## Section 7. Handling and Storage

| | |
|---|---|
| Handling: | Avoid contact with eyes, skin or clothing. Keep container closed. |
| Storage: | Keep container in a cool (2–6°C), dark, UV-protected area. Carefully read and follow all label directions. |

## Section 8. Exposure Controls/Personal Protection

| | |
|---|---|
| Engineering Controls: | Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective occupational exposure limits. |

Personal Protection

| | |
|---|---|
| Eyes: | Where necessary, use a face shield, chemical goggles and provide access to eye/face flushing equipment. |
| Body: | Lab coat. |
| Respiratory: | 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. |
| Hands: | Gloves may be worn when handling this solution. Please refer to glove manufacturer for recommendations. |
| Feet: | Closed-toe shoes of non-porous material with adequate metatarsal coverage should be worn when handling this solution. |

Component Exposure Limits

| | |
|---|---|
| Bacteriophage | No known occupational exposure limits for this component. |
| PBS | (Phosphate Buffered Saline) No known occupational exposure limits for this component. |

## Section 9. Physical and Chemical Properties

| | |
|---|---|
| Odor: | Odorless. |
| Color: | Clear/opalescent. |
| Physical State and Appearance: | Liquid. |
| Molecular Weight: | Not applicable. |
| Molecular Formula: | Not applicable. |
| pH: | 7.2 – 7.5 |
| Boiling/Condensation Point: | The lowest known value is 99.9°C (211.8°F) (WATER). |
| Melting/Freezing Point: | May start to solidify at –0.1°C (31.8°F) based on data for WATER. |
| Vapor Pressure: | Not available. |
| Vapor Density (vs. air): | Not available. |
| Density (vs. water): | 1.005–1.007 g/cm3 |
| Decomposition Temperature: | Not available. |
| Evaporation Rate: | (0.36 (WATER) compared to (n-BUTYL ACETATE=1) |
| Solubility: | Soluble in Water. |
| Viscosity: | Not available. |

## Section 10. Stability and Reactivity

| | |
|---|---|
| Stability and Reactivity: | Stable when used appropriately. |
| Conditions of Instability: | Not available. |
| Incompatibility with Various Substances: | Not available. |
| Rem/Incompatibility: | Not available. |
| Hazardous Decomposition Products: | Not available. |
| Hazardous Polymerization: | Will not occur. |

## Section 11. Toxicological Information

| | |
|---|---|
| Toxicity: | Acute oral toxicity (LD50): 3,000 mg/kg [Rat] (Phosphate Buffered Saline). Acute toxicity of the vapor (LC50): > 42,000 mg/m3 1 hour(s) [Rat] (Phosphate Buffered Saline). |
| Chronic Effects on Humans: | None |
| Acute Effects on Humans: | None |
| Irritancy: | Draize Test: Not a skin irritant (Phosphate Buffered Saline). |
| Sensitization: | Not available. |
| Carcinogenic Effects: | This material is not known to cause cancer in animals or humans (Phosphate Buffered Saline). |
| Toxicity to Reproductive System: | Not available. |
| Teratogenic Effects: | Not available. |
| Mutagenic Effects: | Not available. |

## Section 12. Ecological Information

| | |
|---|---|
| Ecotoxicity: | Not toxic. |
| BOD5 and COD: | Not available. |
| Toxicity of the Products of Biodegradation: | The product itself and its products of degradation are not toxic. |

## Section 13. Disposal Considerations

| | |
|---|---|
| EPA Waste Number: | Not available. |
| Treatment: | Material does not have an EPA Waste Number and is not a listed waste, however consultation with a permitted waste disposal site (TSD) should be accomplished. Always contact a permitted waste disposal (TSD) to assure compliance with all current local, state, and Federal Regulations. |

## Section 14. Transportation Information

| | |
|---|---|
| DOT Classification: | Not applicable. |
| TDG Classification: | Not available. |
| IMO/IMDG Classification: | Not applicable. |
| ICAO/IATA Classification: | Not applicable. |

## Section 15. Regulatory Information

U.S. Federal Regulations: This product is intended solely for use as a food additive in accordance with 21CFR172.785. Components of this product are not listed on the TSCA Inventory or TSCA Inventory status cannot be confirmed.

CERCLA and SARA Regulations (40 CFR 355, 370, 372): This product does not contain any chemicals subject to reporting requirements of SARA Section 313.

| | | |
|---|---|---|
| State Regulations: | Pennsylvania RTK: | Not applicable |
| | Massachusetts RTK: | Not applicable |
| | New Jersey: | Not applicable |
| Illinois: | Not applicable | |
| Michigan: | Not applicable | |
| Minnesota: | Not applicable | |
| Louisiana: | Not applicable | |
| California prop. 65: | Not applicable. | |

Date document prepared: November 30, 2009

## Section 16. Other 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.