

# DEPARTMENT OF HEALTH AND HUMAN SERVICES

## Food and Drug Administration

### 21 CFR Part 172

[Docket No. 2002F–0316] (formerly 02F–0316)

## Food Additives Permitted for Direct Addition to Food for Human Consumption; Bacteriophage Preparation

**AGENCY:** Food and Drug Administration, HHS.

**ACTION:** Final rule.

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**SUMMARY:** The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of a bacteriophage preparation on ready-to-eat meat and poultry products as an antimicrobial agent against *Listeria monocytogenes*. This action is in response to a petition filed by Intralytix, Inc.

**DATES:** This rule is effective [*insert date of publication in the Federal Register*]. Submit written or electronic objections and requests for a hearing by [*insert date 30 days after date of publication in the Federal Register*]. See section VII of this document for information on the filing of objections. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of certain publications in new 21 CFR 172.785 as of [*insert date of publication in the Federal Register*].

**ADDRESSES:** You may submit objections and requests for a hearing, identified by Docket No. 2002F–0316 (formerly 02F–0316), by any of the following methods:

### *Electronic Submissions*

Submit electronic objections in the following ways:

- Federal eRulemaking Portal: <http://www.regulations.gov>. Follow the instructions for submitting comments.
- Agency Web site: <http://www.fda.gov/dockets/ecomments>. Follow the instructions for submitting comments on the agency Web site.

### *Written Submissions*

Submit written objections in the following ways:

- FAX: 301–827–6870.
- Mail/Hand delivery/Courier [For paper, disk, or CD–ROM submissions]:  
Division of Dockets Management (HFA–305), Food and Drug Administration,  
5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

To ensure more timely processing of objections, FDA is no longer accepting objections submitted to the agency by e-mail. FDA encourages you to continue to submit electronic objections by using the Federal eRulemaking Portal or the agency Web site, as described in the *Electronic Submissions* portion of this paragraph.

*Instructions:* All submissions received must include the agency name and Docket No(s). and Regulatory Information Number (RIN) (if a RIN number has been assigned) for this rulemaking. All objections received will be posted without change to <http://www.fda.gov/ohrms/dockets/default.htm>, including any personal information provided. For detailed instructions on submitting objections, see the “Objections” heading of the **SUPPLEMENTARY INFORMATION** section of this document.

*Docket:* For access to the docket to read background documents or objections received, go to <http://www.fda.gov/ohrms/dockets/default.htm> and

insert the docket number, found in brackets in the heading of this document, into the “Search” box and follow the prompts and/or go to the Division of Dockets Management, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

**FOR FURTHER INFORMATION CONTACT:** Raphael A. Davy, Center for Food Safety and Applied Nutrition (HFS-265), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, 301-436-1272.

**SUPPLEMENTARY INFORMATION:**

**I. Background**

In a notice published in the **Federal Register** of July 22, 2002 (67 FR 47823), FDA announced that a food additive petition (FAP 2A4738) had been filed by Intralytix, Inc., c/o Lewis & Harrison, 122 C St. NW., suite 740, Washington, DC 20001, now represented by Keller & Heckman LLP, 1001 G St., NW., suite 500 West, Washington, DC 20001. The petition proposed to amend the food additive regulations to provide for the safe use of a mixture of bacteriophages<sup>1</sup> (phages) as an antimicrobial agent against *Listeria monocytogenes* (*L. monocytogenes*) on foods, including fresh meat, meat products, fresh poultry, and poultry products. On December 18, 2003, the petitioner amended the petition to limit the petitioned use to ready-to-eat (RTE) meat and poultry products only.<sup>2</sup>

The food additive consists of a mixture of equal proportions of six individually purified phages. The petitioner’s rationale for incorporating multiple phages in one formulation is to minimize the possibility of *L. monocytogenes* developing a resistance to the additive. Each phage in the additive is specific against various *L. monocytogenes* strains, including those strains known to be associated with foodborne illness (e.g., *L. monocytogenes*

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<sup>1</sup> Bacteriophages are viruses that infect bacteria only.

<sup>2</sup> Ready-to-eat products, as used in this final rule, are defined in 9 CFR 430.1.

strains, serotypes 1/2a, 4b and 1/2b). The phages are lytic<sup>3</sup> double-stranded DNA phages. The petitioner has characterized each phage with respect to physical properties and other appropriate identifying factors (e.g., host range, structural protein profile, and DNA sequence of complete genome<sup>4</sup>).

In the manufacturing process, each phage contained in the additive is separately produced using a strain of *L. monocytogenes* that can serve as a host to the specific phage. The host *L. monocytogenes* strain is first cultured in microbiological media and the specific phage is added to the culture when a specified cell density is achieved. After phage multiplication, which results in lysis (destruction) of host cells, the phage is purified by use of multiple filtration steps (to remove bacteria and their components). The six phages produced by this process are then blended in phosphate buffered saline solution to formulate the additive. The six phages contained in the additive have been deposited with the American Type Culture Collection<sup>5</sup> (ATCC).

The phage preparation will be used as an antimicrobial agent to control *L. monocytogenes* in the production of RTE meat and poultry products. The phage preparation is directly sprayed on the surface of the RTE food articles at a level of approximately 1 milliliter (mL) of the preparation per 500 square centimeters (cm<sup>2</sup>) of food surface area just prior to packaging.

## **II. Determination of Safety**

Under the general safety standard in section 409 of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348), a food additive cannot be approved for a particular use unless a fair evaluation of the data available to FDA

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<sup>3</sup> Lytic bacteriophages lyse (destroy) their host bacteria as a normal part of their life cycle without integrating into the host genome.

<sup>4</sup> Genome means the genetic content of a cell or virus.

<sup>5</sup> ATCC is a nonprofit bioresource center that maintains deposits of bacteria and bacteriophages among other biological materials. Their primary mission is to acquire, authenticate, preserve, develop, and distribute biological material.

establishes that the additive is safe for that use. FDA's food additive regulations (21 CFR 170.3(i)) define safe as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."

In evaluating the safety of the petitioned substance, FDA considered the following factors in determining the safety of the proposed food additive use: (1) The safety of the six phages constituting the food additive; (2) the safety of potential residues from *L. monocytogenes* used in the manufacture of the food additive and the need for limits related to their levels; (3) whether undesirable genes are potentially carried by the food additive; and (4) the need for additional identity and safety specifications.

#### *A. Safety of the Petitioned Use of the Phage Preparation*

Phages infect only bacteria, rather than mammalian or plant cells.<sup>6</sup> Moreover, phages are ubiquitous and humans are routinely exposed to them at high levels through food, water and the environment without adverse effect.<sup>7</sup> However, the petitioner's bacteriophages are specific to *L. monocytogenes* only. Phages also are a part of the normal microbial population of the human gut.<sup>8</sup> Therefore, FDA concludes that the food additive under consideration does not present a toxicological concern for use in food as proposed by the petitioner based upon the explanations provided in the following sections (Refs. 1 and 3).

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<sup>6</sup> T.D. Brock and M.T. Madigan, 1998, *Biology of Microorganisms*, 5th edition; Prentice-Hall, Inc., Inglewood Cliffs, NJ.

<sup>7</sup> Bergh, O., K.Y. Borsheim, G. Bratbak, and M. Heldal, 1989, High abundance of viruses found in aquatic environments, *Nature*, vol. 340 (10): 467–468.

<sup>8</sup> Breitbart et al., 2003; *Journal of Bacteriology* 185 (20): 6220–6223.

*B. Safety Evaluation of Potential Residue Components From L. monocytogenes*

FDA considered the possibility that the proposed food additive may contain *L. monocytogenes* components as residues from use of the organism as host for phage multiplication in the manufacturing process. Such residues may include the toxin Listeriolysin O (LLO). Potential residues of *L. monocytogenes* other than LLO do not present a safety concern (Ref. 1). Based on our review of scientific literature on the pathogenicity of *L. monocytogenes* (Ref. 1), FDA finds that LLO is the only substance known to be toxic that may potentially be present as a residue in this food additive after the manufacturing process.

LLO was not detected in the finished food additive within the assay limits of detection of 5 hemolytic units<sup>9</sup> (HU)/ml, and the petitioner provided information on the purification process used in the production of the food additive as additional assurance that LLO would not be present at detectable levels in the finished food additive. Nevertheless, the agency has calculated a worst-case exposure to LLO from consumption of food products treated with the phage preparation. Assuming LLO is present at a maximum level of 5 HU/ml in the additive, the worst-case exposure to LLO for males aged 20 years or more that consume RTE foods treated with the additive at the maximum intended use level is 52 HU/person/day (HU/p/d) at the mean and 104 HU/p/d at the 90th percentile. Males aged 20 years or more represent the worst-case scenario because this population group consumes the highest amount of food intended to be treated with the additive (Ref. 2). In this safety evaluation, FDA reviewed all available information on the identity, toxicity, and the

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<sup>9</sup> 1 HU of LLO is equal to one nanogram of protein (as reported in Geoffroy, C. et al. 1987, Purification, Characterization, and Toxicity of the Sulfhydryl-Activated Hemolysin Listeriolysin O from *Listeria monocytogenes*, Infection and Immunity, vol. 55(7): pp. 1641–1646).

stability of LLO. Even if LLO were present at the level of 5 HU/ml, this level does not present a toxicological concern for the following reasons (Ref. 1):

#### 1. Inactivation of LLO by Cholesterol

The toxicity of LLO has been shown to be significantly reduced (by as much as 200- to 2000-fold) following pre-incubation of LLO with added cholesterol *in vitro* (Ref. 1). Since the phage preparation will be used on meat and poultry products and these products normally contain significant (milligram) amounts of cholesterol, then any residual amounts of LLO at levels no greater than 5 HU/ml that may be present in the additive are likely to be inactivated by the cholesterol.

#### 2. pH and LLO Activity

Studies show that LLO activity is lost or significantly decreased in acidic (low pH of less than 4) environments (Ref. 1). Residual amounts of LLO, if present, are likely to be inactivated by the low pH (less than 4) within the human stomach.

#### 3. Inactivation of Orally Consumed LLO by Human Defense Mechanisms

*In vivo* studies demonstrate that both normal intestinal microflora and cell-mediated immunity reactions in the intestines inhibit LLO (Ref. 1). These defense mechanisms provide some protection against low incidental oral exposures to LLO (no greater than 5 HU/ml). Additionally, at these levels, LLO is expected to be rapidly and irreversibly degraded by proteolytic enzymes that may be presented in the diet or in the stomach. Thus, LLO at these residual levels would not pose a toxic threat to humans.

Considering all of the above factors, FDA concludes that potential residues of LLO that may be found in the food additive are negligible (5 HU/ml or less)

and do not pose a safety concern for the use of the additive as an antimicrobial agent on RTE meat and poultry products.

Although LLO was not detected in the food additive, the agency concludes that a specification is necessary to ensure that LLO is not present in detectable amounts to ensure the purity and safe use of the petitioned food additive. Thus, the agency is including in this regulation a specification of not more than 5 HU/ml for LLO (the limit of detection for the method).

### *C. Undesirable Genes (Bacterial Toxin Genes) Potentially Carried by Phages*

Lysogenic phages, as opposed to those that are lytic, have the capacity to integrate into the host genome and may facilitate transfer of toxin or drug resistance genes between bacterial cells. FDA has determined that the phages contained in the petitioned food additive are lytic based on the petitioner's information on host lysis characteristics and on genomic analysis of each phage (Ref. 4). Therefore, FDA concludes that the use of this food additive would not result in the spread of toxin or drug genes.

### *D. The Need for Other Specifications*

We are also including specifications for potency, absence of undesirable genes, phage titer<sup>10</sup>, absence of *L. monocytogenes* and other microbiological pathogens, and total organic carbon (Ref. 2). These specifications ensure the identity and safe use of the additive.

## **III. Other Considerations**

FDA recognizes that while this rule is issued under the authority of the Federal Food, Drug, and Cosmetic Act, use of the ingredient must also comply with the Federal Meat Inspection Act or the Poultry Products Inspection Act, which are administered by the U.S. Department of Agriculture (USDA). In

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<sup>10</sup> A term that refers to the number of phage particles per milliliter of phage solution.

particular, those statutes provide that the ingredient must be suitable for its intended use. FDA recognizes that there may be meat or poultry products considered RTE for which use of the additive may not be suitable within the meaning of those statutes. This regulation addresses only the safety standard under section 409 of the Federal Food, Drug, and Cosmetic Act and does not address requirements for suitability administered by the USDA.

#### **IV. Conclusion**

FDA reviewed data in the petition and other available relevant material to evaluate the safety of the use of a phage preparation as an antimicrobial agent against *L. monocytogenes* on RTE meat and poultry products. Based on this information, the agency concludes that the proposed use of the additive is safe. Therefore, the regulations in part 172 (21 CFR part 172) should be amended as set forth in this document.

In accordance with § 171.1(h) (21 CFR 171.1(h)), the petition and the documents that FDA considered and relied upon in reaching its decision to approve the petition are available for inspection at the Center for Food Safety and Applied Nutrition by appointment with the information contact person (see **FOR FURTHER INFORMATION CONTACT**). As provided in § 171.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

#### **V. Environmental Impact**

The agency has previously considered the environmental effects of this rule as announced in the notice of filing for FAP 2A4738 (67 FR 47823). No new information or comments have been received that would affect the agency's previous determination that there is no significant impact on the human environment and that an environmental impact statement is not required.

## **VI. Paperwork Reduction Act of 1995**

This final rule contains no collection of information. Therefore, clearance by the Office of Management and Budget under the Paperwork Reduction Act of 1995 is not required.

## **VII. Objections**

Any person who will be adversely affected by this regulation may file with the Division of Dockets Management (see **ADDRESSES**) written or electronic objections. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents are to be submitted and are to be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Division of Dockets Management between 9 a.m. and 4 p.m., Monday through Friday.

## **VIII. References**

The following references have been placed on display in the Division of Dockets Management (see **ADDRESSES**) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Memorandum dated June 3, 2005, from Division of Petition Review, Toxicology Group I, Tina Walker, to Raphael Davy, DPR, entitled “Safety Review of LMP-102™ as an antimicrobial agent in ready-to-eat foods, fresh meat, meat products, fresh poultry, and poultry products.”

2. Memorandum dated April 11, 2005, from Division of Petition Review, Chemistry Review Group, Hyoung Lee, to Regulatory Group II, R. Davy, entitled “FAP 2A4738 (MATS#1137 M 2.3), Petition for the use of LMP-102™—a mixture of several monoclonal bacteriophages as an antimicrobial agent in ready-to-eat meat and poultry, Submissions of 10/25/04, 1/18/05, 1/25/05, and 2/18/05.”

3. Memorandum dated February 1, 2006, from Division of Petition Review, Toxicology Group I, Tina Walker, to Raphael Davy, DPR, entitled “Addendum to the June 3, 2005 Final Toxicology Memorandum: Additional toxicological evaluation of the potential allergenicity/immunotoxicity of the *Listera* bacteriophage, LMP-102™.”

4. Memorandum dated June 1, 2005, from Division of Biotechnology and GRAS Notice Review, Negash Belay, to Raphael A. Davy, Division of Petition Review, entitled “Revised FAP 2A4738.”

## List of Subjects in 21 CFR Part 172

Food additives, Incorporation by reference, Reporting and recordkeeping requirements.

■ Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 172 is amended as follows:

### **PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION**

■ 1. The authority citation for 21 CFR part 172 continues to read as follows:

**Authority:** 21 U.S.C. 321, 341, 342, 348, 371, 379e.

■ 2. Section 172.785 is added to subpart H to read as follows:

**§ 172.785** *Listeria*-specific bacteriophage preparation.

The additive may be safely used as an antimicrobial agent specific for *Listeria monocytogenes* (*L. monocytogenes*) in accordance with the following conditions:

(a) *Identity*. (1) The additive consists of a mixture of equal proportions of six different individually purified lytic-type (lacking lysogenic activity) bacteriophages (phages) specific against *L. monocytogenes*.

(2) Each phage is deposited at, and assigned an identifying code by, a scientifically-recognized culture collection center, and is made available to FDA upon request.

(3) The additive is produced from one or more cell cultures of *L. monocytogenes* in a safe and suitable nutrient medium.

(b) *Specifications*. (1) The additive achieves a positive lytic result ( $OD_{600} \leq 0.06$ ) when tested against any of the following *L. monocytogenes* isolates available from American Type Culture Collection (ATCC): ATCC 35152 (serogroup 1/2a), ATCC 19118 (serogroup 4b), and ATCC 15313 (serogroup 1/2b). The analytical method for determining the potency of the additive entitled “Determination of Potency of LMP-102™,” dated October 9, 2003, and printed by Intralytix, Inc., is incorporated by reference. The Director of the Office of the Federal Register approves this incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. You may obtain a copy from the Office of Food Additive Safety (HFS-200), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, or you may examine a copy at the Center for Food Safety and Applied Nutrition’s Library, 5100 Paint Branch Pkwy., College Park, MD

20740, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: [http://www.archives.gov/federal\\_register/code\\_of\\_federal\\_regulations/ibr\\_locations.html](http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html).

(2) The mean phage titer of each monophage in the additive is  $1 \times 10^9$  plaque forming units (PFU)/ml. The analytical method for determining phage titer entitled “Method to Determine Lytic Activity/Phage Titer,” dated November 6, 2001, and printed by Intralytix, Inc., is incorporated by reference. Copies are available at locations cited in paragraph (b)(1) of this section.

(3) The phages present in the preparation must not contain a functional portion of any of the toxin-encoding sequences described in 40 CFR 725.421(d). No sequences derived from genes encoding bacterial 16S ribosomal RNA are present in the complete genomic sequence of the phages.

(4) *L. monocytogenes* toxin, listeriolysin O (LLO), is not greater than 5 hemolytic units (HU)/ml. The analytical method for determining LLO entitled “Quantitation of Listeriolysin O Levels in LMP–102™,” dated September 27, 2004, and printed by Intralytix, Inc., is incorporated by reference. Copies are available at locations cited in paragraph (b)(1) of this section.

(5) The additive is negative for *L. monocytogenes*. The modified version of the U.S. Department of Agriculture’s method for determining *L. monocytogenes* entitled “LMP–102™ *Listeria monocytogenes* Sterility Testing,” dated May 24, 2004, and printed by Intralytix, Inc., is incorporated by reference. Copies are available at locations cited in paragraph (b)(1) of this section.

(6) The additive is negative for gram-positive and gram-negative bacteria capable of growing in commonly used microbiological media (e.g., Luria-

Bertani (LB) medium), including *Escherichia coli*, *Salmonella* species and coagulase-positive *Staphylococci*, as determined by the “Method to Determine Microbial Contamination,” dated July 11, 2003, and printed by Intralytix, Inc., is incorporated by reference. Copies are available at locations cited in paragraph (b)(1) of this section.

(7) Total organic carbon (TOC) is less than or equal to 36 mg/kg. The analytical method for determining TOC entitled “Determination of Total Organic Carbon by Automated Analyzer,” dated March 30, 2001, and printed by Intralytix, Inc., is incorporated by reference. Copies are available at locations cited in paragraph (b)(1) of this section.

(c) *Conditions of use.* The additive is used in accordance with current good manufacturing practice to control *L. monocytogenes* by direct application to meat and poultry products that comply with the ready-to-eat definition in 9

CFR 430.1. Current good manufacturing practice is consistent with direct spray application of the additive at a rate of approximately 1 mL of the additive per 500 cm<sup>2</sup> product surface area.

Dated: August 3, 2006.

**Jeffrey Shuren,**

*Assistant Commissioner for Policy.*

[FR Doc. 06-????? Filed ??-??-06; 8:45 am]

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