Listeria monocytogenes Specific Bacteriophages
PC Code 006495

Preliminary Work Plan
Case Number 5091

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I. Introduction

This document is the Environmental Protection Agency's (EPA or the Agency) Preliminary Work Plan (PWP) for *Listeria monocytogenes* Specific Bacteriophages (Case 5091) and is being issued pursuant to 40 CFR §155.50. This document explains what EPA's Office of Pesticide Programs (OPP) knows about *Listeria monocytogenes* specific bacteriophages, highlights anticipated data and assessment needs, identifies types of information that would be especially useful to the Agency in conducting the review, and provides an anticipated timeline for completing the registration review process for *Listeria monocytogenes* specific bacteriophages. As stated in 40 CFR §155.50 the opening of this docket initiates the current cycle of registration review for *Listeria monocytogenes* specific bacteriophages.

A registration review decision is the Agency's determination whether a pesticide meets, or does not meet, the standard for registration in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, which mandates the continuous review of existing pesticides. All pesticides distributed or sold in the United States generally must be registered by the Agency based on scientific data showing that they will not cause unreasonable adverse effects to human health or to the environment when used as directed on product labeling. The registration review program is intended to ensure that, as the ability to assess and reduce risk evolves and as policies and practices change, all registered pesticides continue to meet the statutory standard of no unreasonable adverse effects. Changes in science, public policy, and pesticide use practices will occur over time. Through the registration review program, the Agency periodically re-evaluates pesticides to ensure that as these changes occur, products in the marketplace can continue to be used safely. Information on this program is provided at www.epa.gov/pesticide-reevaluation.

In 2006, the Agency implemented the registration review program pursuant to FIFRA §3(g). The Agency will review each registered pesticide every 15 years to determine whether it continues to meet the FIFRA standard for registration. The regulations governing registration review are provided in 40 CFR part 155, subpart C. The public phase of registration review begins when the initial docket is opened for the case. The docket is the Agency's opportunity to inform the public what it knows about *Listeria monocytogenes* specific bacteriophages and what additional risk analyses and data or information it believes are needed to make a registration review decision on *Listeria monocytogenes* specific bacteriophages.

The Agency encourages all interested stakeholders to review the PWP and to provide comments and additional information that will help the Agency's decision-making process for *Listeria monocytogenes* specific bacteriophages. Interested stakeholders could include the following: environmental nonprofit or interest groups; pesticide manufacturers; agricultural labor or commodity groups; commercial, institutional, residential, and other users of pesticides; or the general public. In addition to general areas on which persons may wish to comment, there are some areas identified in the PWP about which the Agency specifically seeks comments and information.

After reviewing and responding to comments and data received in the docket during this initial comment period, the Agency will develop and commit to a Final Work Plan (FWP) and anticipated schedule for the registration review of the *Listeria monocytogenes* specific bacteriophages case. Additional information on *Listeria monocytogenes* specific bacteriophages can be found in the Agency's public docket (EPA-HQ-OPP-2024-0178) at www.regulations.gov.

This document is organized into five sections: the *Introduction*, which includes this summary and *Listeria monocytogenes* specific bacteriophages case overview; *Use Information*, which describes how and why *Listeria monocytogenes* specific bacteriophages are used and summarizes data on its use, and the associated pesticide product; *Scientific Assessments*, which summarizes the Agency's risk assessments, any revisions, risk conclusions, and any anticipated data needs that will help the Agency's decision-making process for *Listeria monocytogenes* specific bacteriophages; *Guidance for Commentors*, which highlights topics of special interest, additional information and data the Agency should consider prior to issuing a FWP; and, lastly, the *Next Steps* and *Timeline* provides an anticipated timeline for completing the registration review process for *Listeria monocytogenes* specific bacteriophages.

***Listeria monocytogenes* Specific Bacteriophages Registration Review Case Overview**

Pursuant to 40 CFR §155.50, the Agency will initiate a pesticide's registration review by establishing a docket for registration review of *Listeria monocytogenes* specific bacteriophages (Case 5091) and opening it for public review.

This PWP marks the beginning of the current cycle of registration review for *Listeria monocytogenes* specific bacteriophages, with the opening of public docket EPA-HQ-OPP-2024-0178 available at www.regulations.gov. The following list highlights significant events that have occurred during the current cycle of registration review for this case:

- June 2024 – The Agency is now publishing the *Listeria monocytogenes* specific bacteriophages *Preliminary Work Plan* for a 60-day public comment period.

II. Use Information

Bacteriophage (phages) are viruses that infect bacteria. They are ubiquitous, naturally occurring entities found in soil, water, in association with animals including humans, and plants. ln 2006, the Food and Drug Administration (FDA) classified bacteriophages used to kill *Listeria monocytogenes* on cheese as Generally Recognized as Safe (GRAS). In 2007, FDA expanded the GRAS classification of bacteriophages by allowing its use on all food products. *Listeria monocytogenes* specific bacteriophage are host-specific, effecting or attacking only one bacterial species and most frequently one strain of a species. As a pesticide, *Listeria monocytogenes* specific bacteriophages are used as an adjunct or supplement to traditional food contact sanitizing procedures to mitigate *Listeria monocytogenes* in nonfood areas such as floors, walls, and drains in food processing plants and food handling establishments (U.S. EPA, 2008).

The first pesticide product containing *Listeria monocytogenes* specific bacteriophages as an active ingredient was registered by the Antimicrobials Division (AD) of OPP/EPA in 2008. Currently, there is 1 registered product containing *Listeria monocytogenes* specific bacteriophages, an end-use product, with 0.00001% active ingredient. In 2022, the active ingredient was transferred from AD to the Biopesticides and Pollution Prevention Division (BPPD).

Table 1. *Listeria monocytogenes* Specific Bacteriophages Use Information	
Ingredient Name	*Listeria monocytogenes* specific bacteriophages
PC Code	006495
Pesticide Classification	Microbicide
Use Site Locations	Non-Food Contact Surfaces
Application Types	Spray
No. of Registrations	1 FIFRA Section 3 product[1]
Physical Forms	Liquid

III. Scientific Assessments

A summary of the Agency's human health and ecological risk assessments for *Listeria monocytogenes* specific bacteriophages is presented below. Refer to the Appendix for a listing of product analysis, human health assessment, and non-target organism data that support the scientific assessments for this registration review. For further information on the human health and environmental risk assessments, including a summary of data and literature search findings, please see Appendices B and C.

A. Human Health Assessment

Summary of Hazard Characterization

The submitted mammalian toxicology rationale for *Listeria monocytogenes* specific bacteriophages data requirements were found to be complete. The acute oral toxicity data on the existing product containing *Listeria monocytogenes* specific bacteriophages and the provided rationale supported findings of a lack of unreasonable effects to the U.S. population in general when used in accordance with EPA-approved labeling. However, during the Agency's reassessment, data needs were identified in the supporting product chemistry and efficacy data that are needed to address revised standards for bacteriophage active ingredients including additional product characterization and quality control data obtained with contemporary scientific methods/technology. For further details, please see Appendix A.

Summary of Dietary Exposure and Risk Characterization

Based on the nature of the active ingredient in this case, and the currently approved use pattern which restricts use to indoor application in food processing facilities, residues of *Listeria monocytogenes* specific bacteriophages are not expected to persist on food crops or within the environment (including in bodies of water). As a class of microbial organisms, bacteriophage are not expected to pose a drinking water risk when used for pesticidal purposes due to the general lack of permissible host organisms for growth and replication, and factors contributing to rapid degradation including treatment of drinking water. Additionally, other bacteriophage-containing products have been approved by the Agency and given GRAS status from the FDA. Based on the low toxicity, low exposure scenarios, and the safe history of use it has been concluded that exposure to residues of *Listeria monocytogenes* specific bacteriophages in or on food commodities is unlikely and are not expected to cause harm to adults, children, and infants. Please see Appendix B for further details.

[1] FIFRA labels can be obtained from the Pesticide Product Label System (ordspub.epa.gov/ords/pesticides/f?p=PPLS:1)

Food Tolerances

No registered pesticide products containing *Listeria monocytogenes* specific bacteriophage are approved for food use. Therefore, EPA has not required a tolerance or an exemption from the requirement of a tolerance for residues of *Listeria monocytogenes* specific bacteriophage found in or on food.

Summary of Residential and Non-Occupational Exposure and Risk Characterization

Based on the current approved use patterns of *Listeria monocytogenes* specific bacteriophage-containing product, it is unlikely that residues of the active ingredient will persist within food processing facilities post application of other Agency-approved sanitizers. Residues are therefore unlikely to persist on food commodities or in the environment to reach residential areas or result in non-occupational exposure.

Summary of Occupational Exposure and Risk Characterization

Occupational exposure to the active ingredient may occur during preparation and application within food processing facilities, however, required personal protective equipment (PPE) and strict labeling procedures for microbial pest control products significantly reduces the possibility of exposure. Risks from this exposure to this phage are not expected to occupational handlers when product label precautions are followed. Additionally, the current *Listeria monocytogenes* specific bacteriophage-containing product is Toxicity Category IV, which should pose a low hazard to occupational workers if exposed to the product.

Human Incidents

A search of the Office of Pesticide Programs' (OPP) Incident Data System conducted on March 22, 2024, revealed no reported incidents associated with *Listeria monocytogenes* specific bacteriophages. This database contains information dating back to the 1970s and is continuously updated as incidents are reported.

B. Summary of Environmental Risk Assessment

Environmental exposure outside of food treatment facilities is expected to be minimal based on listed use sites, label instructions and environmental background level of bacteriophages. Any residual amount of the bacteriophages released into the environment are expected to be rapidly inactivated and then degraded given the host specificity to *Listeria monocytogenes* species. Waiver rationale satisfied the nontarget organism data requirements. For details see Appendix C.

Ecological Incidents

A search of OPP's Incident Data System conducted on March 22, 2024, revealed zero reported incidents associated with *Listeria monocytogenes* specific bacteriophages. This database contains information dating back to the 1970s and is continuously updated as incidents are reported.

Endangered Species Assessment

EPA has no reasonable expectation for any registered use of *Listeria monocytogenes* specific bacteriophages to cause direct or indirect discernible effects to threatened and endangered species or their designated critical habitat. This is because of the exclusive indoor use and lack of exposure to non-target organisms. Therefore, EPA is making a "No Effect" determination under the Endangered

Species Act (ESA) for all listed species and designated critical habitat for such species and has therefore concluded that consultation with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service under ESA § 7(a)(2) is not required.

C. Anticipated Data Needs

As described in Appendix A, the existing manufacturing process and bacteriophage characterization requirements are not consistent with the screening criteria currently being applied to new bacteriophage-containing products registered within the Biopesticides and Pollution Prevention Division. As a result, certain product chemistry guideline studies are needed to support a comprehensive evaluation of *Listeria monocytogenes* specific bacteriophages. Additionally, guideline requirements from the Antimicrobial Division pertaining to the Phage Stewardship Program, monitoring for phage effectiveness and resistance, and data supporting label claims are also necessary to support continued registration.

Table 2. Data needs for *Listeria monocytogenes* specific bacteriophages

OCSPP Guideline No.	Data Requirement	Test Substance	Use Site(s) Triggering Data Requirement
885.1200	Manufacturing Process	TGAI, EP	All; revised Quality Assurance/ Quality Control (QA/QC) needed. For the product: • Full genome sequencing and analysis: Confirm bacteriophage taxonomy (according to best available information, Order/Family-level taxonomy is adequate), absence of integrase genes and gene fragments, absence of transposase genes and gene fragments, absence of relevant toxin genes. • For formulations in which phage composition may change subject to host bacteria resistance, each new target bacteria must be identified, purified, named, and confirmed via PCR to be identified to the strain level and submitted to a nationally recognized culture collection consistent with OPPTS 885.1250. • For phage production, confirmation of host bacterial strains by PCR should be integrated into the manufacturing process. Additionally, note that the following QA/QC should be included in the manufacturing process for any phage addition/replacement to the phage library: • Confirm absence of integrase genes and gene fragments by lysogeny assay or PCR • Confirm absence of transposase genes and gene fragments ensuring phage are not capable of gene transduction; can be confirmed by PCR (absence of rRNA) • Host specificity assay - plaque assay on lawn of target host necessary +/- controls. Turbid plaque assay protocol should include the following: inoculation contamination check, phage contamination check, bacteria-free lot check, titration check, titration host control plate check, and turbid plaque check

Table 2. Data needs for *Listeria monocytogenes* specific bacteriophages			
OCSPP Guideline No.	**Data Requirement**	**Test Substance**	**Use Site(s) Triggering Data Requirement**
885.1400	Analysis of Samples	TGAI, EP	All; additional test batches needed; revised QA/QC needed (U.S. EPA. 2005a risk assessment); see notes under **Results/Findings** for Manufacturing Process (885.1200) for necessary analyses for each new phage addition/replacement.
n/a	Label revisions	EP	All; label revisions must address: • Minimum potency/viability in PFU/mL • 5-minute contact time should be supported by data (3-log reduction) • Limitation to hard, non-porous, non-food contact surfaces • Specific use sites/surfaces (i.e., no residential areas, limited to commercial, I&I) • Phage treatment is followed by an EPA-registered antimicrobial pesticide • Contact time included in use directions • Method of application consistent with test method

810.2300	Sanitizers for Use on Hard Surfaces – Efficacy Data Recommendations; Protocol review	TGAI, EP	To fulfill product performance (efficacy) data requirements, a protocol must be submitted for Agency review and must include the following details, at minimum: • 3 lots of bacteriophage preparations • Each test lot must be at the lowest limit concentration per AI as indicated by the CSF • 5 test carriers/coupons per lot • A minimum of 5-minute contact must precede treatment with sanitizer • Inclusion of organic soil in the test method • Potency/titer of bacteriophage cocktail • Details of inoculum preparation: 1 x 10^9 ± 0.5 log PFU/ml; Concentration of each test strain was adjusted to 5 x 10^6 -1 x 10^7 PFU • Requisite study controls (neutralization effectiveness confirmation, sterility, etc.) • Identification of phages • Monitoring of phages • Minimum product performance criteria based on proposed claim (e.g., 99.9% for sanitizer) • Include justification for any unique/product-specific testing modifications • 3-log reduction must be demonstrated Monitoring program requirements must include: • Use of reference target bacterial strains as well as submitted test strains in each assay of material from customers. • Consistency of test conditions, including temperature. Phage dynamics can be temperature dependent, so temperature conditions should be standardized. • Description of strains characterization at the production laboratory. Description of the speciation methods, and Q/A involved. Food processing facilities are not likely to want to have their strains speciated, or at least not likely to want the results generally available, so a discussion of how that information would be handled is warranted. • Results of the Bacterial Resistance Monitoring program must be communicated to the Agency should evidence of resistance emerge. • If/When bacteria resistance has been identified and the “phage removal and replacement” strategy is implemented, the Agency must be notified of any proposed changes in the cocktail preparation, and the conditions in which the resistant bacterium was identified. • The registrant must determine the size of the cocktail (usually 1 or 2 phages) and library (sometimes upward of 180 bacteriophages). The Agency must be informed of which phages are included in the initial cocktail, and any modifications (i.e., additions, deletions, substitution) in the formulation. The registrant should provide justifications for any modifications to the cocktail, substantiated by results of the Bacterial Resistance Monitoring program. • There may be multiple formulations of the phage product (considering that resistance to phage may be site specific, have seasonal implications, as well as geographical influences). If so, this must be documented on the CSF as alternate formulations.

IV. Guidance for Commentors

Preliminary Work Plan

During the comment period, anyone may submit relevant data or information for the Agency's consideration. The public is invited to comment on the Agency's PWP. The areas below highlight topics of special interest to the Agency where comments, information and data, or reference to sources of additional information could be of particular use. The Agency will carefully consider all comments, as well as any additional information or data provided in a timely manner, prior to issuing a FWP for this case.

Additional Information

Stakeholders are also specifically asked to provide information and data that will assist the Agency in refining the risk assessments. The Agency is interested in obtaining the following information regarding *Listeria monocytogenes* specific bacteriophages:

i. Confirmation on the following label information:
   - *Sites of application*
   - *Formulations*
   - *Application methods and equipment*
   - *Maximum application rates*
   - *Frequency of application, application intervals, and maximum number of applications*
   - *Geographic limitations on use*

ii. Use or potential use distribution (e.g., acreage and geographical distribution of relevant use sites)

iii. Median and 90th percentile reported use rates from usage data – national, state, and county

iv. Application timing (date of first application and application intervals) – national, state, and county

v. Usage/use information for agricultural and nonagricultural uses

vi. Typical application interval (days)

vii. State or local use restrictions

viii. Monitoring data

ix. Foreign technical registrants not listed above who supply pesticide products containing *Listeria monocytogenes* specific bacteriophages to the U.S. market

Environmental Justice

EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues related to registration review decisions, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical, unusually high exposure to *Listeria monocytogenes* specific bacteriophages compared to the general population or who may otherwise be disproportionately affected by the use *Listeria monocytogenes* specific bacteriophages as a pesticide. Please comment if you are aware of any such issues and can provide information to help the Agency to more fully consider and address potential environmental justice issues.

V. Next Steps and Timeline

A Federal Register Notice will announce the docket opening for the current cycle of registration review for *Listeria monocytogenes* specific bacteriophages and a 60-day comment period for this *Preliminary Work Plan* to provide comments and additional information that will help the Agency's decision-making process for *Listeria monocytogenes* specific bacteriophages. After the 60-day comment period closes, the Agency will review and respond to any comments received in a timely manner, then issue a Final Work Plan for *Listeria monocytogenes* specific bacteriophages. The Agency's final decision on the *Listeria monocytogenes* specific bacteriophages registration review case will occur following satisfaction of the EDSP obligations under FFDCA §408(p).

Table 3. Anticipated Registration Review Schedule for *Listeria monocytogenes* Specific Bacteriophages

Anticipated Activity	**Estimated Month/ Year**
Opening the Docket	
Open Docket and 60-Day Public Comment Period for Preliminary Work Plan	June 2024
Close Public Comment Period	August 2024
Case Development	
Final Work Plan	December 2024
Issue DCI	March 2025
Data Submission	March 2026
Open 60-Day Public Comment Period for Draft Risk Assessments	March 2027
Close Public Comment Period	May 2027
Registration Review Decision and Implementation	
Open 60-Day Public Comment Period for Proposed Registration Review Decision	TBD
Close Public Comment Period	TBD
Registration Review Decision	TBD
*Estimated Total (Years)	TBD

*The anticipated schedule will be revised as necessary (e.g., need arising under the Endocrine Disruptor Screening Program with respect to the active ingredients in this case).

Appendix A –Product Characterization

The product analysis data for *Listeria monocytogenes* specific bacteriophages have been reviewed and found to be supplemental but upgradeable as determined in the initial review (U.S. EPA, 2005a) and listed in Table 4. Since its initial registration, this active ingredient has been transferred from the Antimicrobials Division (AD) to the Biopesticides and Pollution Prevention Division which requires additional microbial pesticide guidelines to be applied to all *Listeria monocytogenes* specific bacteriophage-containing products. Additionally, revised standards have been applied across all new bacteriophage active ingredients which serve to provide additional product characterization and quality control data through contemporary scientific methods/technology and thus should be applied to *Listeria monocytogenes* specific bacteriophages. Finally, the efficacy claims associated with *Listeria monocytogenes* specific bacteriophages are not currently supported by data. These deficiencies and data gaps should be addressed with the submission of additional data in order to support registration review.

Table 4 Summary of Product Analysis Data (40 CFR §158.2120)

Data Requirement	Guideline No.	Results / Findings	MRIDs
Product Identity	885.1100	All bacteriophages within the currently registered product have been adequately characterized. **Classification: Acceptable**	46169301
Manufacturing Process	885.1200	The data submitted in support of the initial registration is acceptable. However, since initial product registration, revised QA/QC standards have been applied to all new bacteriophage-containing products. Therefore, additional QA/QC are needed within a revised manufacturing process. For each new product: • Full genome sequencing and analysis: Confirm bacteriophage taxonomy (according to best available information, Order/Family-level taxonomy is adequate), absence of integrase genes and gene fragments, absence of transposase genes and gene fragments, absence of relevant toxin genes. • For formulations in which phage composition may change subject to host bacteria resistance, each new target bacteria must be identified, purified, named, and confirmed via PCR to be identified to the strain level and submitted to a nationally recognized culture collection consistent with OPPTS 885.1250. • For phage production, confirmation of host bacterial strains by PCR should be integrated into the manufacturing process. For each new phage addition/replacement: • Confirm absence of integrase genes and gene fragments by lysogeny assay or PCR • Confirm absence of transposase genes and gene fragments ensuring phage are not capable of gene transduction; can be confirmed by PCR (absence of rRNA)	46169301

		• Host specificity assay - plaque assay on lawn of target host necessary +/- controls. • Turbid plaque assay, protocol should include the following: inoculation contamination check, phage contamination check, bacteria-free lot check, titration check, titration host control plate check, and turbid plaque check Results of these screening procedures are to be reported within the Analysis of Samples (885.1400) **Classification: Supplemental but upgradable**	
Deposition of a Sample in a Nationally Recognized Culture Collection	885.1250	All bacteriophages within the currently registered product have been deposited within the American Type Culture Collection. **Classification: Acceptable**	46169301
Discussion of Formation of Unintentional Ingredients	885.1300	**Classification: Acceptable**	46169301
Analysis of Samples	885.1400	Additional test batches needed; revised QA/QC needed (U.S. EPA. 2005a risk assessment); see notes under **Results/Findings** for **Manufacturing Process (885.1200)** for necessary analyses for each new phage addition/replacement. **Classification: Supplemental but upgradeable**	46169302
Certified Limits	885.1500	**Classification: Acceptable**	46169302
Color	830.6302	**Classification: Acceptable**	46169303
Physical State	830.6303	**Classification: Acceptable**	46169303
Odor	830.6304	**Classification: Acceptable**	46169303
Stability to Normal and Elevated Temperatures, Metals, and Metal Ions	830.6313	**Classification: Acceptable**	46169303
Storage Stability	830.6317	**Classification: Acceptable**	46169303
pH	830.7000	**Classification: Acceptable**	46169303
Density/Relative Density/Bulk Density (Specific Gravity)	830.7300	**Classification: Acceptable**	46169303

Appendix B – Human Health Risk Assessment

In previous reviews of the active ingredient the Agency had determined that no unreasonable adverse effects to the U.S. population in general, and to infants and children in particular, are expected when the pesticide product containing the active ingredient *Listeria monocytogenes* specific bacteriophages is used in accordance with EPA-approved labeling (U.S. EPA, 2008; U.S. EPA, 2005a). However, a reassessment of the supporting product chemistry and efficacy data as they pertain to this product and its transfer from the Antimicrobials Divisions to the Biopesticides and Pollution Prevention Division indicate the presence of data gaps. In the time since the initial registration of this bacteriophage active ingredient, revised standards have been applied across all new bacteriophage active ingredients which serve to provide additional product characterization and quality control data through contemporary scientific methods/technology and thus should be applied to *Listeria monocytogenes* specific bacteriophages. This new assessment will provide support for the current registration of the *Listeria monocytogenes* specific bacteriophage-containing pesticide product.

Summary of Mammalian Toxicology Data

The mammalian toxicology data for *Listeria monocytogenes* specific bacteriophages have been reviewed and found to be complete. There are no data or rationale which suggests toxicological concerns or hazards associated with the registered active ingredient. Table 5 summarizes the current mammalian toxicology data requirements for *Listeria monocytogenes* specific bacteriophages.

Table 5. Summary of Toxicology Data (40 CFR §158.2140)

Data Requirement	OCSPP Guideline No.	Results / Findings	MRIDs
Acute Oral Toxicity/Pathogenicity	885.3050	Waived based on rationale, review of published literature, and lack of acute oral toxicity associated with the end use product (MRID 45453504) **Classification: Acceptable** **Toxicity Category IV**	U.S. EPA, 2005a
Acute Pulmonary Toxicity/Pathogenicity	885.3150	Waived based on rationale, review of published literature, and lack of acute oral toxicity associated with the end use product (MRID 45453504) **Classification: Acceptable** **Toxicity Category IV**	U.S. EPA, 2005a
Acute Injection Toxicity/Pathogenicity	885.3200	Waived based on rationale, review of published literature, and lack of acute oral toxicity associated with the end use product (MRID 45453504) **Classification: Acceptable** **Toxicity Category IV**	U.S. EPA, 2005a
Hypersensitivity Incidents	885.4300	Waived based on rationale and review of published literature; any hypersensitivity incidents must be reported (refer to test note #3 of 40 CFR § 158.2140(d)) **Classification: Acceptable** **Toxicity Category IV**	U.S. EPA, 2005a
Cell Culture	885.3500	Waived based on rationale and review of published literature **Classification: Acceptable** **Toxicity Category IV**	U.S. EPA, 2005a

Hazard Characterization

The toxicological database is considered complete for characterizing hazard and assessing risk from the active ingredient in this case. *Listeria monocytogenes* specific bacteriophages can be classified as Toxicity Category IV for acute oral, inhalation, eye irritation, and primary dermal irritation (U.S. EPA, 2008). The Agency does not anticipate the need for additional human health studies for this registration review. All data requirements, per 40 CFR §158.2140, have been fulfilled for *Listeria monocytogenes* specific bacteriophages. Hazard and exposure data, Agency risk assessments, and other information on this active ingredient were evaluated against standards established by FIFRA and the Agency's regulations and scientific policies (U.S. EPA, 2005a; U.S. EPA, 2008).

Dietary Exposure and Risk Characterization

Based on the nature of the active ingredient, residues of *Listeria monocytogenes* specific bacteriophages and currently approved use patterns which are restricted to indoor application in food processing facilities, residues are not expected to persist on food crops or within the environment (including in bodies of water). As a class of microbial organisms, bacteriophage are not expected to pose a drinking water risk when used for pesticidal purposes due to the general lack of permissible host organisms for growth and replication, and factors contributing to rapid degradation including treatment of drinking water (Balogh, 2010; Chan, 2013; Salmond, 2015). Additionally, other bacteriophage-containing products have been approved by the Agency and given GRAS (Generally Recognized As Safe) status from the FDA and USDA (U.S. FDA, 2007, 2013a, 2013b, 2014, 2016, 2017, 2018a, 2018b, 2018c, 2019a, 2019b, 2019c; U.S. EPA, 2011, 2015, 2016). Based on the low toxicity, low exposure scenarios, and the safe history of use it has been concluded that exposure to residues of *Listeria monocytogenes* specific bacteriophages in or on food commodities is unlikely and are not expected to cause harm to adults, children, and infants (U.S. EPA, 2005a; U.S. EPA, 2008).

Residential and Non-Occupational Exposure and Risk Characterization

Based on the currently approved use patterns of the *Listeria monocytogenes* specific bacteriophage-containing product, it is unlikely that residues of the active ingredient will persist within food processing facilities post application of other Agency-approved sanitizers. Residues are therefore unlikely to persist on food commodities or in the environment to reach residential areas or result in non-occupational exposure.

Occupational Exposure and Risk Characterization

Occupational exposure to the active ingredient may occur during preparation and application within food processing facilities, however, required PPE and strict labeling procedures for microbial pest control products significantly reduces the possibility of exposure. Workers handling the end-use product containing *Listeria monocytogenes* specific bacteriophage must wear long-sleeved shirt, long pants, shoes, socks, and waterproof or chemical-resistant gloves. A respirator is also required for handlers of microbial active ingredients to reduce the risk of respiratory sensitization. Additionally, rationale including citation of publicly available literature support categorization of current *Listeria monocytogenes* specific bacteriophage-containing product as Toxicity Category IV, indicating low hazard to occupational workers should exposure occur. Risks to occupational handlers are not expected from use of this active ingredient when product label restrictions/precautions are followed.

Overall Human Health Risk Characterization and Conclusion

Based on the toxicity and pathogenicity data and rationale submitted in support of the initial registration of *Listeria monocytogenes* specific bacteriophages there is expected to be low risk of toxicity or pathogenicity to workers and the public. The currently approved indoor-only use pattern of *Listeria monocytogenes* specific bacteriophages for sanitizing surfaces within food processing facilities significantly reduces the risk of exposure to humans through potential residues on food commodities or through dissemination in the environment.

Literature Search Findings

To support registration review, the Biopesticides and Pollution Prevention Division (BPPD) conducts searches of the literature and incident databases to determine if there are any reports of adverse effects that might change risk conclusions or change knowledge of the state of the science for *Listeria monocytogenes* specific bacteriophages. Searches conducted for *Listeria monocytogenes* specific bacteriophages are described below.

Human Health Results:

A search of potentially relevant scientific literature was performed using the PubMed and Google Scholar search engines. The searches were designed to survey all relevant publications from the last 10 years with keywords containing "bacteriophage," "bacteriophages," "*Listeria monocytogenes* specific bacteriophage," "*Listeria monocytogenes* specific bacteriophages," "*Listeria monocytogenes*," and "bacteriophages," alone and combined with the terms "human" and "infection" or "infectivity," "toxicity," "pathogen," "pathogenicity," "allergy," "allergic," or "sensitization," in various iterations. These searches yielded several to hundreds of results varying according to term broadness and can be generally described as studies pertaining to *Listeria monocytogenes* genetics and detection or bacteriophages as biocontrol tools for suppression of *Listeria monocytogenes* contamination. Survey of the results did not yield studies that suggested concern for human health or risk of toxicity or pathogenicity. An incident search performed for *Listeria monocytogenes* specific bacteriophages using the Agency's pesticide incident database system returned no incident reports.

No additional information was gained from these searches that would alter the BPPD's understanding of the current state of the science for any potential effects of *Listeria monocytogenes* specific bacteriophages on humans or nontarget organisms.

Appendix C – Environmental Risk Assessment

Summary of Nontarget Organism Data

Table 6. Summarizes the current nontarget organism data requirements and rationale supporting registration review of *Listeria monocytogenes* specific bacteriophages. The submitted rationale is adequate, complete, and supports the data requirements.

Table 6. Summary of Nontarget Organism Data (40 CFR §158.2150)

Data Requirement	Guideline No.	Results / Findings	MRIDs
Avian oral toxicity	885.4050	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Avian inhalation toxicity/pathogenicity	885.4100	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Wild mammal toxicity/pathogenicity	885.4150	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Freshwater fish toxicity/ pathogenicity	885.4200	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Freshwater invertebrate toxicity/pathogenicity	885.4240	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Estuarine/Marine fish testing Estuarine/Marine invertebrate testing	885.4280	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Nontarget plant testing	885.4300	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)
Nontarget insect testing	885.4340	Provided rationale describes that the active ingredient is exclusively used inside food production facilities.	(U.S. EPA, 2005b)

Risk Characterization

All nontarget organism and environmental fate data necessary to meet the standard for *Listeria monocytogenes* specific bacteriophages were satisfied via the acceptance of waiver rationales. The submitted waiver rationale explained that the bacteriophages will only be used inside food processing facilities on nonfood surfaces. After use of the bacteriophages, routine sterilization and cleaning processes will eliminate the bacteriophages, and any amount minor residual released into the environment will not change environmental background levels given the ubiquity of these bacteriophages in the nature. Any residual amount of the bacteriophages released into the environment are expected to be rapidly inactivated and then degraded given the host specificity to *Listeria monocytogenes* species (U.S. EPA, 2005b).

The nontarget organism data requirements were satisfied via acceptable rationale and there are no data gaps. No additional information was identified that would alter the current state of the science for this active ingredient. For further information on the ecological risk assessment, including summary of data see Table 6 above and literature search findings below.

Literature Search Findings

To support registration review, the Biopesticides and Pollution Prevention Division (BPPD) conducts searches of the literature and incident databases to determine if there are any reports of adverse effects that might change risk conclusions or change knowledge of the state of the science for *Listeria*

monocytogenes specific bacteriophages. Searches conducted for *Listeria monocytogenes* specific bacteriophages are described below.

Ecological Results:

A literature search was conducted using the Web of Science Core Collection, the default database within the Web of Science system, with the terms " *Listeria monocytogenes* Bacteriophages " and "avian," "mammals," "plants," "insects," and "aquatic organisms," which returned 1,087 results. The results discussed the discovery of *Listeria* bacteriophages, research on *Listeria* bacteriophages, uses in food production facilities, and medical uses of bacteriophages. None of the results discussed any effects on nontarget organisms. An ecological incident search was performed for *Listeria monocytogenes* specific bacteriophages using the incident data search system in prism, and no incident reports were returned.

No additional information was gained from these searches that would alter the BPPD's understanding of the current state of the science for any potential effects of *Listeria monocytogenes* specific bacteriophages on humans or nontarget organisms.

Appendix D – Endocrine Disruptor Screening Program (EDSP)

The Federal Food Drug and Cosmetic Act (FFDCA) §408(p) requires EPA to develop a screening program to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." (21 U.S.C. 346a(p)). In carrying out the Endocrine Disruptor Screening Program (EDSP), FFDCA section 408(p)(3) requires that EPA "provide for the testing of all pesticide chemicals," which includes "any substance that is a pesticide within the meaning of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), including all active and pesticide inert ingredients of such pesticide." (21 U.S.C. 231(q)(1) and 346a(p)(3)). However, FFDCA section 408(p)(4) authorizes EPA to, by order, exempt a substance from the EDSP if the EPA "determines that the substance is anticipated not to produce any effect in humans similar to an effect produced by a naturally occurring estrogen." (21 U.S.C. 346a(p)(4)).

The EDSP initiatives developed by EPA in 1998 includes human and wildlife testing for estrogen, androgen, and thyroid pathway activity and employs a two-tiered approach. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid pathways. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance and establish a dose-response relationship for any adverse estrogen, androgen, or thyroid effect. If EPA finds, based on that data, that the pesticide has an adverse endocrine-related effect on humans, FFDCA § 408(p)(6) also requires EPA, "… as appropriate, [to] take action under such statutory authority as is available to the Administrator … as is necessary to ensure the protection of public health." (21 U.S.C. 346a(p)(6))[2].

Between October 2009 and February 2010, EPA issued Tier 1 test orders/data call-ins (DCIs) for its first list of chemicals ("List 1 chemicals") for EDSP screening and subsequently required submission of EDSP Tier 1 data for a refined list of these chemicals. EPA received data for 52 List 1 chemicals (50 pesticide active ingredients and 2 inert ingredients). EPA scientists performed weight-of-evidence (WoE) analyses of the submitted EDSP Tier 1 data and other scientifically relevant information (OSRI) for potential interaction with the estrogen, androgen, and/or thyroid signaling pathways for humans and wildlife.[3]

In addition, for FIFRA registration, registration review, and tolerance-related purposes, EPA collects and reviews numerous studies to assess potential adverse outcomes, including potential outcomes to endocrine systems, from exposure to pesticide active ingredients. Although EPA has been collecting and reviewing such data, EPA has not been explicit about how its review of required and submitted data for these purposes also informs EPA's obligations and commitments under FFDCA section 408(p). Consequently, on October 27, 2023, EPA issued a Federal Register Notice (FRN) providing clarity on the applicability of these data to FFDCA section 408(p) requirements and near-term strategies for EPA to further its compliance with FFDCA section 408(p). This FRN, entitled *Endocrine Disruptor Screening Program (EDSP): Near-Term Strategies for Implementation' Notice of Availability and Request for Comment* (88 FR 73841) is referred to here as EPA's EDSP Strategies Notice. EPA also published three

[2] For additional details of the EDSP, please visit https://www.epa.gov/endocrine-disruption.
[3] Summarized in *Status of Endocrine Disruptor Screening Program (EDSP) List 1 Screening Conclusions*; EPA-HQ-OPP-2023-0474-0001; https://www.regulations.gov/document/EPA-HQ-OPP-2023-0474-0001

documents supporting the strategies described in the Notice:

- *Use of Existing Mammalian Data to Address Data Needs and Decisions for Endocrine Disruptor Screening Program (EDSP) for Humans under FFDCA Section 408(p)*;
- *List of Conventional Registration Review Chemicals for Which an FFDCA Section 408(p)(6) Determination is Needed;* and,
- *Status of Endocrine Disruptor Screening Program (EDSP) List 1 Screening Conclusions* (referred to here as List 1 Screening Conclusions).

The EDSP Strategies Notice and the support documents are available on www.regulations.gov in docket number EPA-HQ-OPP-2023-0474. As explained in these documents, EPA is prioritizing its screening for potential impacts to the estrogen, androgen, and thyroid systems in humans, focusing first on conventional active ingredients. Although EPA voluntarily expanded the scope of the EDSP to screening for potential impacts to the estrogen, androgen, and thyroid systems in wildlife, EPA announced that it is not addressing this discretionary component of the EDSP at this time, considering its current focus on developing a comprehensive, long-term approach to meeting its Endangered Species Act obligations (See EPA's April 2022 ESA Workplan[4] and November 2022 ESA Workplan Update[5]). However, EPA notes that for 35 of the List 1 chemicals (33 active ingredients and 2 inert ingredients), Tier 1 WoE memoranda[6] indicate that available data were sufficient for FFDCA section 408(p) assessment and review for potential adverse effects to the estrogen, androgen, or thyroid pathways for wildlife. For the remaining 17 List 1 chemicals, Tier 1 WoE memoranda made recommendations for additional testing. EPA expects to further address these issues taking into account additional work being done in concert with researchers within the EPA's Office of Research and Development (ORD).

As discussed in EPA's EDSP Strategies Notice and supporting documents, EPA will be using all available data to determine whether additional data are needed to meet EPA's obligations and discretionary commitments under FFDCA section 408(p). For some conventional pesticide active ingredients, the toxicological databases may already provide sufficient evaluation of the chemical's potential to interact with estrogen, androgen, and/or thyroid pathways and EPA will generally not need to obtain any additional data to reevaluate those pathways, if in registration review, or to provide an initial evaluation for new active ingredient applications. For instance, EPA has endocrine-related data for numerous conventional pesticide active ingredients through either a two-generation reproduction toxicity study performed in accordance with the current guideline (referred to here as the updated two-generation reproduction toxicity study; OCSPP 870.3800 - Reproduction and Fertility Effects) or an extended one-generation reproductive toxicity (EOGRT) study (OECD Test Guideline 443 - Extended One-Generation Reproductive Toxicity Study). In these cases, EPA expects to make FFDCA 408(p)(6) decisions for humans without seeking further estrogen or androgen data. However, as also explained in the EPA's EDSP Strategies Notice, where these data do not exist, EPA will reevaluate the available data for the conventional active ingredient during registration review to determine what additional data, if any, might be needed to confirm EPA's assessment of the potential for impacts to estrogen, androgen, and/or thyroid pathways in humans. For more details on EPA's approach for assessing these endpoints, see EPA's EDSP Strategies Notice and related support documents.

[4] https://www.epa.gov/system/files/documents/2022-04/balancing-wildlife-protection-and-responsible-pesticide-use_final.pdf
[5] https://www.epa.gov/system/files/documents/2022-11/esa-workplan-update.pdf
[6] https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and

Also described in the EPA's EDSP Strategies Notice is a framework that represents an initial approach by EPA to organize and prioritize the large number of conventional pesticides in registration review. For conventional pesticides with a two-generation reproduction toxicity study performed under a previous guideline (i.e., an updated two-generation reproduction toxicity study or an EOGRT is not available), EPA has used data from the Estrogen Receptor Pathway and/or Androgen Receptor Pathway Models to identify a group of chemicals with the highest priority for potential data collection (described in EPA's EDSP Strategies Notice as Group 1 active ingredients). For these cases, although EPA has not reevaluated the existing endocrine-related data, EPA has sought additional data and information in response to the issuance of EPA's EDSP Strategies Notice to better understand the positive findings in the ToxCast™ data for the Pathway Models and committed to issuing DCIs to require additional EDSP Tier 1 data to confirm the sufficiency of data to support EPA's assessment of potential adverse effects to the estrogen, androgen, and/or thyroid pathways in humans and to inform FFDCA 408(p) data decisions. For the remaining conventional pesticides (described in EPA's EDSP Strategies Notice as Group 2 and 3 conventional active ingredients), EPA committed to reevaluating the available data to determine what additional studies, if any, might be needed to confirm EPA's assessment of the potential for impacts to endocrine pathways in humans.

Although EPA has prioritized conventional active ingredients as presented in EPA's EDSP Strategies Notice, EPA is planning to develop similar strategies for biopesticide and antimicrobial pesticide (*i.e.*, nonconventional) active ingredients and will provide public updates on these strategies, when appropriate. At this time, EPA is making no findings associated with the implementation of EDSP screening of *Listeria monocytogenes* specific bacteriophages. Such issues will be addressed in future updates by EPA on its strategies for implementing FFDCA section 408(p).

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