



US Environmental Protection Agency Office of Pesticide Programs

**BIOPESTICIDES REGISTRATION ACTION DOCUMENT
(*Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae*
pv. *tomato* specific Bacteriophages)
(Chemical PC Codes 006449 and 006521)**

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**U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division**

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Environmental Fate and Effects Risk Assessment

Zigfridas Vaituzis, Ph.D.

Health Effects Risk Assessment

John Kough, Ph.D.

Carl Etsitty, M.S.

Registration Support

Leonard Cole, M.S.

Biopesticides Registration Action Document Team Leader

Leonard Cole, M.S.

Office of General Counsel

Chris Kaczmarek

I. Executive Summary

OmniLytics is seeking a Section 3 registration for a microbial pesticide to control bacterial diseases on tomato and pepper plants. The active ingredients are a mixture of bacteriophages specific to control the bacteria *Xanthomonas campestris pathovare (pv.)* and *Pseudomonas syringae pathovare tomato*. The end-use product, Agriphage, is specifically formulated to suit each user's needs to control the strains of *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* in the fields. The company selects the mixture from the company's library of characterized phages of *Xanthomonas campestris pv.* and *Pseudomonas syringae pv. tomato*. The percent of active ingredient is 0.046%.

II. Overview

A. Use Profile

- **Active Ingredient:** *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages.
- **Trade and Other Names:** AgriPhage
- **OPP Chemical Code:** **006449 *Xanthomonas campestris pv. vesicatoria*** and **006521 *pseudomonas syringae pv. tomato***
- **Basic Manufacturer:** OmniLytics
P.O. Box 4296
Logan, Utah 84323-4296
- **Type of Pesticide:** Microbial
- **Uses:** Tomatoes and Peppers
- **Target Pest(s):** The bacteria *Xanthomonas campestris pv. vesicatoria* and *pseudomonas syringae pv. tomato*

B. Regulatory History

The company, AgriPhi, first petitioned the Agency on March 31, 1994 for an experimental use permit (EUP) and temporary exemption from the requirement of a tolerance for the bacteriophages specific for the bacteria *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato*. The EUP was granted on August 29, 1994. The company under the new name OmniLytics has applied for a full commercial use section 3 registration for the control of *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* on tomato and pepper plants. The active ingredient is a mixture of bacteriophages specific for the bacteria *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato*.

III. Science Assessment

A. Physical and Chemical Properties Assessment

The data submitted in support of the product identity requirements for *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are sufficient for the proposed use patterns of the microbial pesticide.

1. Product Identity and Mode of Action (MRID 465754-01)

Phages are ubiquitous, naturally occurring viruses that are found in soil, food, and drinking water. The mode of action of these phages is lysogenic. They attack the host bacteria causing the bacterial wall to rupture, thus killing the bacteria. Bacteriophages are typically named based on the host bacteria and the named “pathovar” or pv. The active ingredients, *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are host specific bacteriophages effective only against the bacteria *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* that attack both tomatoes and peppers. These bacteriophages were first collected from tomatoes and peppers infected with these bacteria on diseased fruits.

These bacteriophages are tested for efficacy against certain strains of bacteria using aseptic cross streaking methods, and once efficacy is confirmed, the phages are purified and are stored in a cryogenic library to insure purity is maintained. These stored bacteriophages are highly specific for the targeted bacterial hosts. Steps are taken to test for efficacy of stored phages.

The bacteria *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* attack tomatoes and peppers causing the plant disease called bacterial spot and bacterial speck respectively. Bacterial spot is a severe disease to tomatoes and peppers. Damage to the plants includes leaf and fruit spots which result in reduced yields, defoliation, and sun scalded fruits. Further, the fruits develop blisters and ultimately rot. Bacterial speck of tomatoes causes lesions and holes on green fruits and dark brown to black flakes on ripe fruits. *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages directly attack the bacterial spot and tomato speck bacteria killing them.

The Agency has reviewed the manufacturing process for the bacteriophages and has determined that this process is acceptable.

Table 1: Product Identity & Manufacturing Process for *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific Bacteriophages (MRID 465754-01)

Guideline	Study	Result
151-10 *885.1100	Product Identity	ACCEPTABLE. The active ingredient is a naturally-occurring virus that attacks and kills bacteria.
151-11 885.1200	Manufacturing Process	ACCEPTABLE. No toxicological impurities are associated with the active ingredient.
151-12 885.1300	Discussion of Formation of Unintentional Ingredients	ACCEPTABLE. Products are produced by an integrated system. Description of production process is sufficient.
151-13 885.1400	Analysis of Samples	ACCEPTABLE. Samples were analyzed prior to mixing with final inert ingredients. Quantification of phage counts were done by plaque counting.
151-15 885.1500	Certification of limits	ACCEPTABLE. Certified limits are within OPPTS guidelines.
151-16	Analytical Method	ACCEPTABLE. Serial dilutions of <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> specific bacteriophages are plated and incubated. Phage counts are determined by plaque counting after incubation.

*OPPTS Guidelines

2. Physical and Chemical Properties Assessment (MRID 465754-02)

Guideline data requirements 40 CFR Part §158.740(a) for color, physical state, odor, storage stability, and pH for this product are described below (Table 2).

Table 2. Physical and Chemical Properties Assessment of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* specific Bacteriophages (MRID 465754-02)

Guideline	Study		MRID #
63-2 *830.6302	Color	Light to medium brown	465754-02
63-3 *830.6303	Physical state	Liquid	465754-02
63-4 *830.6304	Odor	None to slight	465754-02
63-17 *830.6317	Storage Stability	Stable for more than 1 year when stored in a cool dry place away from sunlight and direct heat.	465754-02
63-12 *830.7000	pH	6.5-6.8	465754-02

*OPPTS Harmonized Test Guidelines

B. Human Health Assessment

1. Food Clearances/Tolerances

This is the first proposed Section 3(c)(7)(5) conditional registration of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas* pv. *syringae* *tomato* specific bacteriophages.

There is a reasonable certainty that no harm is likely to result from exposure to the active ingredient. This includes all anticipated dietary exposures for which there is reliable information. As such, an exemption from the requirement of a food tolerance for residues of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages is being established concomitant with the conditional registration (40 CFR Part 180). Below is the toxicology assessment and discussion of other factors covered under the Food Quality Protection Act (1996), which led to the decision to grant an exemption from tolerance.

The Agency has considered this organism in light of the safety factors identified in the Food Quality Protection Act (FQPA) and has made a determination of reasonable certainty of no harm to the US population in general and to infants and children in particular. Due to the ubiquitous nature of this organism and the fact that similar phages are consumed daily in the diet, no adverse effects are expected from exposure to this organism.

2. Toxicology Assessment

a. Acute Oral Toxicity (Waived) MRID 465754-03

The literature cited demonstrate that bacteriophages are ubiquitous; prevalent in soil and drinking water. Humans consume bacteriophages in their daily diets. (Eayre *et al.*, 1995) In light of the fact that the cited literature states that these phages are non-toxic and non-pathogenic, and they are host specific to bacteria, the acute oral toxicity study was waived. (US EPA Memo From Etsitivity to Cole, 2005)

b. Acute Pulmonary Toxicity/Pathogenicity (Waived) MRID 465754-03

The literature cited demonstrate that bacteriophages are ubiquitous; prevalent in soil and drinking water. Humans consume bacteriophages in their daily diets. Humans ingest phages daily with no adverse effects reported. In light of the fact that the cited literature states that phages are non-toxic and non-pathogenic, and they are host specific to bacteria, the acute pulmonary toxicity/pathogenicity study was waived. (Etsitivity, 2005)

c. Acute Injection Toxicity/Pathogenicity (Waived) MRID 465754-03

The literature cited demonstrate that bacteriophages are ubiquitous; prevalent in soil and drinking water. Humans consume bacteriophages in their daily diets. Humans ingest phages daily with no adverse effects reported. (Whitman, *et al.*, 1971) Further, phages are host specific to bacteria only. The cited literature indicated that phages would pose no adverse effects to humans; therefore, the acute injection toxicity/pathogenicity study was waived. (Etsitivity, 2005)

d. Acute Dermal Toxicity (Waived) MRID 465754-03

The literature cited demonstrate that humans are exposed to phages daily. They are naturally occurring ubiquitous viruses that are prevalent in soil, drinking water and food. The literature cited indicate that dermal exposure to humans would pose no adverse effects; therefore, the acute dermal study was waived. (Etsitivity, 2005)

e. Acute Inhalation Toxicity (Waived) MRID 465754-03

Since bacteria are the hosts of bacteriophages and are ubiquitous non-toxic and non-pathogenic organisms, the cited literature demonstrate the exposure via inhalation would pose no adverse effects. Moreover, humans are exposed daily to phages, and they consume phages in their daily diets. Further, cited literature indicate that phages are found in the human gut, and no adverse effects have been reported. Therefore, the acute inhalation toxicity study was waived. (Etsitivity, 2005)

f. Primary Eye Irritation (Waived) MRID 465754-03

The literature cited demonstrate that bacteriophages are ubiquitous; prevalent in soil and drinking water. Humans consume bacteriophages in their daily diets. Humans are exposed to phages daily with no adverse effects reported. Further,

phages are host specific to specific strains of bacteria only. Therefore, the primary eye irritation study was waived. (Etsitty, 2005)

g. Primary Dermal Irritation (Waived) MRID 465754-03

The literature cited demonstrate that bacteriophages are ubiquitous naturally occurring organisms. Humans are exposed daily to these organisms without non adverse effects. Since these phages are non-toxic and non-pathogenic to humans and animals, the primary dermal irritation study was waived. (Etsitty, 2005)

Table 3: Tier I - Acute Mammalian Toxicity of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages

Guideline	Study	Toxicity Category	Results
152-30 *870.1100	Acute oral toxicity	N/A	WAIVER GRANTED. This study was waived based on literature cited proving that humans and animals consume phages daily in their diets with no adverse effects.
152-32 885.3150	Acute pulmonary toxicity/ pathogenicity	N/A	WAIVER GRANTED. The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic to humans.
152-33 885.3200	Acute injection toxicity/ pathogenicity	N/A	WAIVER GRANTED. The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic to humans.
152-31 870.1200	Acute dermal toxicity	N/A	WAIVER GRANTED. The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic to humans.

Xanthomonas campestris pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages

152-32 870.1300	Acute inhalation toxicity	N/A	WAIVER GRANTED. The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic to humans.
152-35 870.2400	Primary eye irritation	N/A	WAIVER GRANTED. Literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic to humans.
152-34 870.2500	Primary dermal irritation	N/A	WAIVER GRANTED. The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic to humans.

h. Hypersensitivity Study MRID 465754-03

This study was waived due to non-toxicity and non-pathogenicity. Cited literature indicated that the acute oral, acute dermal, acute pulmonary, injection toxicity/pathogenicity, and dermal irritation all were non-toxic to humans. There have been no known reports of hypersensitivity incidents. (Etsitty, 2005)

i. Immune Response MRID 465754-03

The cited literature demonstrated that *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages are non-toxic, non-pathogenic, or infective to humans and animals. Therefore, this study was waived. (Etsitty, 2005)

Table 4: Acute Mammalian Toxicity of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages

Guideline	Study	Toxicity Category	Comments
152-36 *870.2600	Hypersensitivity	N/A	The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic, infective, or pathogenic.
152.38 870.2500	Immune Response	N/A	WAIVER GRANTED. The literature cited demonstrated that <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> specific bacteriophages are nontoxic or pathogenic.

*OPPTS Guidelines

j. Tissue Culture

This study was waived based on cited literature and the fact that *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages have been deemed non-toxic.

k. Effects on the Immune and Endocrine Systems

EPA is required under section 408(p) of the FFDCA as amended by FQPA to develop a screening program substances (including all pesticide active and other ingredients) “may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate.” *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages are not known endocrine disruptors nor are they related to any class of known endocrine disruptors. Consequently, endocrine-related concerns did not adversely impact the Agency’s safety finding for *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages. *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific

bacteriophages and similar phages have been deemed non-toxic based on published literature.

Tier II and Tier III Studies

Tier II and Tier III Studies were not required based on the non-toxicity of the active ingredients *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages.

3. Dietary Exposure and Risk Characterization

Due to the proposed use of *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae tomato* specific bacteriophages on tomatoes and peppers, residues may be present on these commodities. However, negligible to no risk is expected for the general population, including infants and children, because *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae tomato* specific bacteriophages are non-toxic and non-pathogenic.

4. Occupational and Residential Exposure and Risk Characterization

a. Non-occupational Residential, School and Day Care Exposure, and Risk Characterization

Xanthomonas campestris pv. vesicatoria and *Pseudomonas syringae pv. tomato* specific bacteriophages will be applied to agricultural fields. Since these application sites are not generally located near residential areas, there will be little opportunity for non-occupational exposures to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages. Moreover, in the unlikely event of such exposure, no harm would be expected due to the active ingredient's non-toxicity.

b. Occupational Exposure and Risk

Potential worker and pesticide handler exposure to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are not expected to pose any undue risk. Appropriate Personal Protective Equipment (PPE) and a Restricted Entry Interval (REI) of 12 hours are required to mitigate any potential risks to workers and pesticide handlers. PPE for workers and handlers consists of long-sleeved shirt, long pants, shoes, socks, waterproof gloves, and a filtering respirator.

The primary routes of exposure for mixer/loaders and applicators would be dermal and/or inhalation exposure. The acute pulmonary toxicity/pathogenicity cited literature submitted in support of the registration demonstrated that *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato*

specific bacteriophages are non-toxic and non-pathogenic. As such, the risks anticipated for occupational exposure are considered minimal.

5. Drinking Water Exposure and Risk Characterization

Bacteriophages similar to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are found in drinking water, and humans ingest these bacteriophages on a daily basis. Further the product may only be applied to agricultural fields of tomatoes and peppers. Accordingly, application of this pesticide to tomatoes and peppers is not expected to increase drinking water exposure to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages. Furthermore, any material that is consumed through drinking water would pose no risk for the general population, including infants and children, due to the pesticide's non-toxicity and non-pathogenicity.

6. Acute and Chronic Dietary Risks for Sensitive Subpopulations Particularly Infants and Children

There is a reasonable certainty that no harm to the U.S. population, including infants and children, will result from aggregate exposure to residues of *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages due to the use as a microbial pest control agent. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are non-toxic and non-pathogenic, and non-infective to mammals. Cited literature indicates that all types of phages are consumed daily by humans in food and drinking water with no adverse effects. (Summers, *et. al.*, 2001) Accordingly, exempting *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages from the requirement of a tolerance is considered safe and poses no significant risks.

7. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

The potential for aggregate exposure should be adequately mitigated if label instructions are followed.

a. Dermal

Non-occupational exposure should be minimal due to the use sites (agricultural fields), and minimal potential for wind dispersal of foliar applied spray products is expected.

Occupational dermal exposure is limited by use of the required PPE and REI.

b. Oral

Oral exposure would occur primarily from eating treated produce. Additionally, such bacteriophages are ingested daily by humans in food and water; therefore, negligible to no risk is expected, because *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages demonstrated no pathogenicity or toxicity potential.

c. Inhalation

The potential for non-occupational inhalation exposure to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages residues are unlikely, because the potential use sites are agricultural. The greatest likelihood of inhalation exposure would occur in an occupational setting among applicators. However, as demonstrated in the public literature, and due to the fact that humans consume phages daily in their diets, *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are non-infective, pathogenic, or toxic. Despite the benign nature of the active ingredient, the Agency requires that all workers exposed to microbial pesticides must wear a dust/mist filtering respirator. As such, the risks anticipated for inhalation exposure are considered minimal.

8. Cumulative Effects

Section 408(b)(2)(D)(v) of the FFDCA requires the Agency to consider the cumulative effect of exposure to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages and to other substances that have a common mechanism of toxicity. Phages are ubiquitous, naturally-occurring viruses that attack specific bacteria found in soil, water and are consumed by animals including humans with no adverse effects having been ever reported, no cumulative exposure is expected. Further, phages including *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are inactivated within 24-48 hours after application and the inactivated phages are biodegradable, no cumulative exposure with other compounds is expected.

9. Determination of Safety for U.S. Population, Infants and Children

There is a reasonable certainty that no harm to the U.S. population, including infants and children, will result from aggregate exposure to residues of *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages due to the use as a microbial pest control agent. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages are non-toxic, non-pathogenic, and non-infective to mammals. Accordingly, exempting *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* specific bacteriophages from the requirement of a tolerance is considered safe and poses no significant risks.

FDCA section 408(b)(2)(C) provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database on toxicity and exposure, unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety), which often are referred to as uncertainty factors, are incorporated into EPA risk assessment either directly or through the use of a margin of exposure analysis or by using uncertainty (safety) factors in calculating a dose level that poses no appreciable risk. Actual exposures to adults and children through diet are expected to pose no adverse effects due to the fact that humans consume phages in their diets daily with no reported adverse effects. Thus, the Agency has determined that an additional margin of safety for infants and children is unnecessary.

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

Effects on non-target species

The registrant submitted written data waiver justifications for the OPPTS Microbial Pesticide Guideline requirements primarily based on the ubiquity and host specificity of bacteriophages. The registrant's waiver justifications are supported with specific literature citations relating to the specificity of phages for bacterial hosts, the ubiquity of phages in the environment including water, soil, crops and processed foods, and in the human body without adverse effects. The waiver requests were reviewed by the Agency and the results of the assessment are presented here in both tabular (Table 5) and more detailed descriptive format. (MRID No. 460938-02)

Table 5: Tabular results of waiver requests

Guideline	Study	Status	MRID Number
885.4050	Avian oral toxicity/pathogenicity	The submitted data waiver justification is sufficient for hazard assessment. No hazard to avian species is apparent.	460938-02
885.4150	Wild mammal testing	The human health assessment data are sufficient to make a no apparent hazard finding.	460938-02
885.4200	Freshwater fish toxicity/pathogenicity	The submitted data waiver justification is sufficient for hazard assessment. Negligible or no exposure or hazard to freshwater fish is anticipated.	460938-02
885.4240	Freshwater aquatic invertebrate toxicity/pathogenicity	The submitted data waiver justification is sufficient for hazard assessment. Negligible or no exposure or hazard to aquatic invertebrate species is apparent.	460938-02
885.4280	Estuarine and marine animal testing	Testing not required because of low to no exposure to the estuarine/marine environment.	N/A
885.4340	Nontarget Insect toxicity/pathogenicity	The submitted data waiver justification is sufficient for hazard assessment. Negligible to no hazard to non-target insect species is anticipated.	460938-02
885.4380	Honey Bee toxicity/pathogenicity	The submitted data waiver justification is sufficient for hazard assessment. Negligible to no hazard to honeybees is anticipated.	460938-02
Endangered Species Act (ESA)	Endangered Species Impact Assessment	The Agency performed an ESA assessment and determined that no adverse effects are expected to endangered species.	None

Hazard Characterization for Terrestrial Wildlife

Avian Hazard Assessment

The literature citations submitted with the data waiver justifications (MRID 460938-02) demonstrate that bacteriophages are ubiquitous; prevalent in soil, water, in association with animals, including humans, and plants. Therefore, avian species are exposed to phages throughout their natural environment. The literature also shows that phages are host specific to bacteria. That is, specific bacteriophages attack only one bacterial species and most frequently only one strain of a bacterial species. Agriphage is specific to *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* bacteria and therefore would not cause adverse effects to non-target organisms including avian species. Furthermore, phages are inactivated 24-48 hours after application to plants or soil and are biodegradable. The phage end-products are broken down and recycled as nutrients for soil inhabitants. The data provided indicate that no avian hazard is expected from the proposed uses of Agriphage. (Vaituzis, 2004)

Non-target Insect and Honey Bee Hazard Assessment

The literature citations submitted with the data waiver justifications (MRID 460938-02) demonstrate that bacteriophages are ubiquitous; prevalent in soil, water, and in association with animals, including humans, and plants. Therefore, insects are exposed to phages throughout their natural environment. The literature also shows that phages are host-specific to bacteria. That is, specific bacteriophages attack only one bacterial species and sometimes only one strain of a bacterial species. Agriphage is specific to *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* bacteria and therefore would not cause adverse effects to insects, including honeybees. Furthermore, phages are inactivated 24-48 hours after application to plants or soil and are biodegradable. The phage end-products are broken down and recycled as nutrients for soil inhabitants. The data provided indicates that no adverse effects to insects are expected from the proposed uses of Agriphage. (Vaituzis, 2004)

Wild Mammal Hazard Assessment

These data are required only when the human health data are not sufficient for wild mammal hazard assessment. The human health hazard assessment data are sufficient to make a no apparent hazard to wild mammals finding.

Hazard Characterization for Aquatic Wildlife

Freshwater Fish Hazard Assessment

The literature citations submitted with the data waiver justifications (MRID 460938-02) demonstrate that bacteriophages are ubiquitous; prevalent in soil, *water*, and in association with animals, including humans, and plants. Therefore, freshwater fish are exposed to phages throughout their natural environment. The literature also shows

that phages are host-specific to bacteria. That is, specific bacteriophages attack only one bacterial species and most frequently only one strain of a bacterial species. Agriphage is specific to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* bacteria and therefore, would not cause adverse effects to freshwater fish species. Moreover, phages are inactivated 24-48 hours after application to plants or soil and are biodegradable. The phage end-products are broken down and recycled as nutrients for soil inhabitants. Agriphage is for terrestrial use only (control of bacterial spot of tomato/pepper) and thus exposure to freshwater fish would be minimal and limited to run-off from fields adjacent to water. The data provided indicate that the proposed uses of Agriphage would not pose a hazard for freshwater fish. (Vaituzis, 2004)

Freshwater Aquatic Invertebrate Hazard Assessment

The literature citations submitted with the data waiver justifications (MRID 460938-02) demonstrate that bacteriophages are ubiquitous; prevalent in soil, water, and in association with animals, including humans, and plants. Therefore, aquatic invertebrates are exposed to phages throughout their natural environment. The literature also shows that phages are host-specific to bacteria. That is, specific bacteriophages attack only one bacterial species and most frequently only one strain of a bacterial species. Agriphage is specific to *Xanthomonas campestris pv. vesicatoria* and *Pseudomonas syringae pv. tomato* bacteria and therefore would not cause adverse effects to aquatic invertebrates. Moreover, phages are inactivated 24-48 hours after application to plants or soil and are biodegradable. The phage end products are broken down and recycled as nutrients for soil inhabitants. Agriphage is for terrestrial use only and thus exposure to aquatic invertebrates would be minimal and limited to run-off from fields adjacent to water. The data provided indicates that the proposed uses of Agriphage would not pose a hazard for aquatic invertebrates. (Vaituzis, 2004)

Estuarine and Marine Animal Hazard Assessment

These data are conditionally required only when the product is intended for direct application to the estuarine and marine environment, or is expected to enter this environment in significant concentrations because of the intended use or mobility pattern. Since this product is not intended for direct use to estuarine and marine environments, should there be any run-off to adjacent fields it would not be expected to be in significant concentrations. Agriphage is for terrestrial use only and thus exposure to aquatic invertebrates would be minimal and limited to run-off from fields adjacent to water. Accordingly, testing for Agriphage is not required because of low to no exposure to estuarine/marine ecosystems. (Vaituzis, 2004)

Endangered species considerations

Since bacteria are the hosts for bacteriophages, it follows that they would be found where bacteria are abundant. In fact, phages are ubiquitous in the environment, known since the early 1900's and isolated from all types of bacteria from almost any aquatic or terrestrial habitat where bacteria can exist. Agriphage is composed of bacteriophages isolated from plant debris and soil. Bacteriophages are host-specific for bacteria, with specific bacteriophages attacking only one bacterial species and most frequently only one strain of a bacterial species. Agriphage is intended for use to control bacterial spot of tomato/pepper and is specific to the target bacterial pathogens, *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*. Moreover, phages are inactivated 24-48 hours after application to plants or soil and are biodegradable. The phage end-products are broken down and recycled as nutrients for soil inhabitants. Agriphage is for terrestrial use only and thus exposure to aquatic organisms would be minimal and limited to run-off from fields adjacent to water. (Vaituzis, 2004)

The reviewed literature citations provided by the registrant demonstrating the ubiquity of bacteriophages in the environment, host-specificity for bacteria, rapid inactivation and biodegradability of Agriphage, and lack of published reports of adverse effects on eucaryotic wildlife indicate that exposure to Agriphage will have no measurable deleterious effects on endangered species. That is, there is a “no effect” finding in connection with any endangered/threatened species listed by the U.S. Fish and Wildlife Service (USFWS). (Vaituzis, 2004)

2. ENVIRONMENTAL FATE

Agriphage is composed of bacteriophages isolated from plant debris and soil that are used to control bacterial spot of tomato/pepper and are specific to the target bacterial pathogens, *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*. Bacteriophages (phages) are ubiquitous, naturally occurring viruses found in soil, water, and in association with animals, including humans, and plants. In general, bacteriophages are bacteria species-specific and usually strain specific. They have been isolated from almost any habitat where bacteria exist and have been studied since the early 1900's. Phages are inactivated 24-48 hours after application to plants or soil and are biodegradable. The phage end products are broken down and recycled as nutrients for soil inhabitants. (Vaituzis, 2004)

ENVIRONMENTAL RISK ASSESSMENT SUMMARY

The Agency has performed an environmental risk assessment based on the literature citations provided by the registrant and has determined that the proposed uses of Agriphage will have no adverse effects on avian species, wild mammals, fish, aquatic invertebrates, and insects, including the honeybee. Agriphage does not pose a hazard to wildlife at the proposed use rates, so there is also a “no effect” finding in connection with any endangered/threatened species listed by the USFWS.

IV. ACTIONS REQUIRED BY REGISTRANT/DATA GAPS

The Registrant must verify minimal bioactivity for each batch expressed as plaque forming units (PFU) and the bacterial strain tested. The manufacturing process should indicate where lysozyme is added to the product. An accounting of the mixture of *Xanthomonas campestris* pv. *vesicatoria* specific bacteriophages must be provided to the Agency. This accounting must include the library strain designation for each phage used and the specific designation for each bacterial host isolate used for production of that particular phage. The same phage library reference and production bacterial host information must also be provided for each of the *Pseudomonas syringae* pv. *tomato* specific bacteriophage used in an Agriphage product. The bacterial strain (specified by library reference number) must also be tracked along with the calculated plaque forming units (PFU) for each phage in the mixture. While a system for screening newly isolated phage before entry into the phage library, the procedure for adding new host bacteria into the host library to be used in the phage production system should be explained in detail and submitted to the Agency. A PCR based screen to address the presence of lysogeny associated traits is under development by AgriPhi/OmniLytics and its addition to the QC procedures for phage selection is anticipated.

In order to prevent the accidental mixing of incorrect combinations of phage/bacteria, the company must have procedures in place to perform transfers/inoculations with only one combination at a time in any one place. This could be accomplished by several means, one of which is color coding the phage/host bacteria labels on flasks and inoculum, restricting transfers/inoculations to single site and controlling movement of materials to that site by a QC step. This same method would also be appropriate for the final mixing stage where individual phage batches are mixed to make AgriPhage final product.

V. Regulatory Position

Pursuant to FIFRA Section 3(c)(7)(C), the Agency may conditionally register a pesticide containing an active ingredient not contained in any currently registered pesticide for a period reasonably sufficient for the generation and submission of required data (which are lacking because a period reasonably sufficient for generation of the data has not elapsed since the Agency first imposed the data requirement) on condition that by the end of such period the Agency receives such data and the data do not meet or exceed risk criteria enumerated in regulations issued under this Act, and on such other conditions as the Agency may prescribe. The Agency recently imposed the aforementioned data requirement after the registrant had submitted the application for section 3 registration. Due to this fact, the registrant has not had sufficient time to generate these data thereby meeting this criterion.

1) The registrant must submit a PCR based screen within 6 months of this registration, and

2) The company must maintain Quality Control procedures and report these procedures to the Agency if requested.

TABLE 6: Use Site Conditional Registration - *Xanthomonas campestris pv. vesicatoria and Pseudomonas syringae pv. tomato* specific bacteriophages

***Xanthomonas campestris pv. vesicatoria and Pseudomonas syringae pv. tomato* specific bacteriophages**

Agriphage: tomatoes and peppers.

Official date registered:

VI. References Cited:

A. BPPD Technical Support Documents

References: Biopesticides and Pollution Prevention Division Memoranda and Reviews

- April 13, 2004 Review of Data Waiver Requests for the Registration of a New Microbial Pesticide: *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages. Memorandum from Hilary Hill and Zigfridas Vaituzis to Leonard Cole. US EPA/OPP/BPPD
- September 29, 2005 Review of Data and Data Waiver Requests for the Registration of a New Microbial Pesticide: *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific bacteriophages. Memorandum from Carl Etsitty and John Kough to Leonard Cole. US EPA/OPP/BPPD

B. MRID Citation References

- 460938-02 Jones, J. Data Waiver Requests for Ecological Toxicity Studies. Unpublished study prepared by Delta Analytical.
- 465754-01 Jackson, L., and Braverman, M. Product Identity and Manufacturing Process. Unpublished study prepared by IR-4 Project.
- 465754-02 Jackson, L. and Braverman, M. Physical and Chemical Properties. Unpublished study prepared by IR-4 Project.
- 465754-03 Jackson, L. and Braverman, M. Data Waiver Requests for Toxicity Studies. Unpublished study prepared by IR-4 Project.

C. Public Literature

Eayre, C.G., J.A. Bartz and D.E. Concelmo. Bacteriophages of *Erwinia carotovora* and *Erwinia ananas* isolated from freshwater lakes. *Plant Disease*. Vol. 79, No. 8, August 1995, pp. 801-804.

Summers, William C. Bacteriophage therapy. *Annual Review Microbiology*. Vol. 55, 2001, pp. 437-451.

Whitman, P.A. and R.T. Marshall. Isolation of psychrophilic bacteriophages-host systems from refrigerated food products. *Applied Microbiology*. Vol. 22, No. 2, August 1971, pp 220-223.