UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Ecological Risk Assessment for the New Active Ingredients Bacteriophages active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* contained in the Agri-phage Nut and Stone Fruit formulated product, produced by OmniLytics Inc. proposed for FIFRA Section 3 registration

Action Code Case:	00149952
Submission Number:	1063978
EPA File Symbol:	67986-RN
Active Ingredient Type:	Microbial
PC Codes:	101111 to 101114
MRID Numbers:	51027902, 51027905, 51027911

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I. Executive Summary

Bacteriophages active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* are new active ingredients. The phages are proposed to control bacterial diseases caused by the bacteria *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas*

arboricola pv. *corylina, Pseudomonas syringae* pv. *syringae* that are commonly known as bacterial spot, canker, and blast on nuts and stone fruit.

Agriphage Nut and Stone Fruit is a multiphage mixture which selectively attaches, lyses, and destroys the cells of the bacteria; *Pseudomonas syringae* pv. *syringae, Xanthomonas arboricola* pv. *corylina, Xanthomonas arboricola* pv. *juglandis,* and *Xanthomonas arboricola* pv. *pruni*. Agriphage Nut and Stone Fruit contains only lytic phages which naturally decompose when their hosts are no longer active or alive.

All nontarget organisms may be exposed during foliar and chemigation application methods, or when consuming or coming into contact with soil, trees, insects, and drainage water where bacteriophages active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* are present. Nontarget plants may be exposed during spray applications of bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* through contact with spray on the treated area, spray drift, and via runoff.

The phages are not expected to persist in nature because they require a host for survival and replication, and should be eliminated and/or return to background levels by the time runoff reaches water bodies, because the phages will die when their hosts are no longer active or living. Bacteriophages cannot infect mammalian cells but specifically target bacteria. This specificity is highly refined and each phage will only attack one species or, in some cases, a single strain of bacterium (Hanlon, 2007). The host specificity study submitted by the applicant documented the inability of the active ingredient phages to infect and replicate in a variety of bacteria, such as *Pseudomonas aeruginosa, Escherichia coli, and Salmonella enterica* (MRID 51027905). Given this host specificity there are no expected adverse effects for nontarget organisms associated with the use of bacteriophages active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae.*

EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-target species exposed to bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* as a result of the proposed labeled applications. Therefore, a "No Effect" determination is made for direct and indirect effects to federally listed threatened and endangered ('listed') species and their designated critical habitats resulting from the proposed uses of bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae,* as labeled.

II. Introduction

Phages are antibacterials that can be applied to living organisms and selectively target bacteria in a very narrow host range (Chan, 2013) A bacteriophage is a type of virus that may infect bacterial cells. A specific bacteriophage exists for each specific bacterium (Hraiech et al., 2015), and each kind of phage specifically targets only certain bacteria as its host (Gorski et al., 2006).

An important benefit of using phages is that the microbiome of an organism is not disrupted because of the high selectivity inherent to phages. Phage activity is such that only a few strains within a bacterial species are affected (Chan, 2013). Bacteriophages cannot infect mammalian cells, but they do specifically target bacteria. This specificity is highly refined and each phage will only attack one species or in some cases a single strain of bacterium (Hanlon, 2007).

Phages have been used to treat humans, mammals, avian species, fruit trees, honey bees, and other animals. Phages of *Paenibacillus larvae* are used to treat honey bees because of their specificity (Yost et al., 2016). Phage therapy has been used to decrease the presence of the human pathogen *Campylobacter jenjuni*, in live broiler chickens prophylactically and therapeutically, reducing colonization levels significantly (Wagenaar et al., 2005). *Pseudomonas syringae* is a pathogen that is important for stone-fruit orchard growers. Pathovars that are related to *P. syringae* cause bacterial canker of the *Prunus* family which includes the important fruit trees of cherry, plum, peach, and apricot. (Hulin et al., 2018).

Prunus spp. and *Juglans* spp. are frequently infected by *Xanthomonas arboricola*. These infections have been reported as more common over the past ten years as compared to previous decades. Furthermore, *X. arboricola*, pv. *pruni* and pv. *juglandis*, are the pathogens that cause the diseases bacterial canker and spot and bacterial blight on stone fruits, almond, and walnut (Lamichhane, 2014). Therefore, the bacteriophages active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* may be effectively used to combat these bacterial pathogens. Bacteriophages are ubiquitous and are isolated from nature and all phages used in AgriPhage-Nut & Stone Fruit have been isolated from infected fruit and nut trees. It is most common to isolate bacteriophages from actual infected areas of a fruit or nut tree because phages maintain bacterial levels at an equilibrium. Thus, it is known bacteriophages are active against *Pseudomonas syringae* pv. *syringae, Xanthomonas arboricola* pv. *corylina, Xanthomonas arboricola pv. juglandis,* and *Xanthomonas arboricola* pv. *pruni* naturally exist in each geographic region affected by bacterial spot, canker, and blast on nuts and stone fruit.

The EPA previously concluded that the submitted rationale was adequate to meet the data requirements for the bacteriophage active against *Xanthomonas citri* subsp. *citri* to control citrus canker. Phage therapy has also been employed as a tool to treat bacterial fruit tree diseases previously, with no adverse effects expected for any nontarget organisms (MRID 51027911). The weight of evidence clearly demonstrates that phage therapy is widely used in medicine and agriculture to prevent bacterial diseases, and that the host range of bacteriophages used are very limited to the bacteria that are being targeted.

EPA also previously concluded that effects on the environment are not expected to occur from the registered use of the bacteriophages active against *X. campestris* pv. *vesicatoria* and *P. syringae* pv. tomato (US EPA, 2015). Additionally, the Agency made a "No Effect" determination under the Endangered Species Act (ESA) for direct and indirect effects to listed species and their designated critical habitats. Furthermore, registered bacteriophages of *X. campestris* pv. *vesicatoria* and *P. syringae* pv. *tomato* only infect pepper and tomato plants, and bacteriophages are specific to a particular bacterium, bacteriophages of *X. campestris* pv. *vesicatoria* and *P. syringae* pv. tomato are unable to infect mammalian cells (US EPA, 2015).

Mode of Action

Agriphage Nut and Stone Fruit is a multiphage mixture which selectively attaches, lyses, and destroys the cells of the bacteria; *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*. Agriphage Nut and Stone Fruit contains only lytic phages which naturally decompose when their hosts are no longer active or alive.

Use and Usage

Agriphage Nut & Stone Fruit bactericide for the biological control for bacterial spot, canker, and blast can be used as a preventive measure to protect growing leaf and fruit tissue or when conditions are conducive to heavy disease pressure, and as a curative when the first disease symptoms are visible on all stone and nut fruits. The pesticide may be applied as a foliar or chemigation application with repeated application as needed or weekly at a maximum rate of 2 quarts per 50 gallons of dilution water.

III. Nontarget Organism Exposure

All nontarget organisms may be exposed during foliar and chemigation application methods.

A. Terrestrial Environments

The maximum exposure will result from foliar and or chemigation 2 quarts per 50 gallons of water per acre. The estimated environmental concentration (EEC) for terrestrial organisms for the maximum application rate is based on the average enumeration from the analysis of samples for the end use product (7.4×10^{10} PFU/ml plaque forming units) (MRID 51027902). This amount multiplied by the volume of product used, which is 1892.7 ml (2 quarts/acre) and equals 1.4×10^{14} PFU. This number divided by 189,271 ml (50 gallon minimum dilution) equals 7.4×10^{8} PFU/ml, which is the estimated concentration of the a.i. in the spray, and used as the most conservative the EEC.

1. Birds, Mammals, and Nontarget Plants

These species may be directly exposed during routine pesticide applications. Birds and wild mammals may also be exposed when consuming or coming into contact with soil, trees, insects, and drainage water where bacteriophage active against *Xanthomonas arboricola* pv. *pruni*,

Xanthomonas arboricola pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* is present. Nontarget plants may be exposed during spray applications of bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* and via runoff.

2. Nontarget Insects

Nontarget insects may be exposed during spray and soil applications directly, via runoff, or by consuming plant parts, seeds, or soil. Pollinators may be exposed during or after application of the end use product and when visiting plants to gather nectar and/or pollen.

B. Aquatic Environments

Freshwater and estuarine/marine fish and invertebrates, and aquatic plants may be exposed during intense runoff events that occur soon after application of bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* or through drift when applications occur near water bodies.

The phages are not expected to persist in nature because they require a host for survival and replication and should be eliminated or return to background levels by the time runoff reaches water bodies, because the phages will die when their hosts are no longer active or living. These concentrations are expected to quickly decline and most cases of exposure of aquatic organisms to bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae* are not likely to be different from natural background levels.

IV. Summary of Nontarget Effects Data

Table 1 provides the status of the data requirements as published in 40 CFR § 158.2150 for ecological risk assessment. Scientific rationale has been submitted to satisfy data requirements for avian oral, wild mammals, freshwater fish, freshwater invertebrates, nontarget insects, nontarget plant, and honey bee testing. The information provided is sufficient to satisfy the Tier I nontarget organism data requirements for ecological risk assessment for the active ingredient. Further testing of nontarget organisms at higher tiers is not required for the proposed label uses.

Table 1. Summary of rationale submitted to comply with nontarget organism data requirements published in 40 CFR § 158.2150 for support of the registrations of products containing Bacteriophage active against *Xanthomonas arboricola* pv. *pruni, Xanthomonas arboricola* pv. *juglandis, Xanthomonas arboricola* pv. *corylina, Pseudomonas syringae* pv. *syringae.*