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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Human Health Risk Assessment of Bacteriophages active against *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*, New Active Ingredients, in 67986-RN AgriPhage Nut & Stone Fruit Proposed for Registration and an Associated Petition Requesting a Tolerance Exemption

Submission Number(s):	1063978, 1063985
Action Code Case No(s):	00293012, 00292018
EPA Reg. No(s). or File Symbol(s):	67986-RN (EP)
Active Ingredient Name:	Bacteriophages active against *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*
PC Codes:	101111, 101112, 101113, 101114
Tolerance Exemption Petition:	1F8907
MRID(s):	51027901, 51027902, 51027903, 51027904, 51027905, 51027906, 51027907, 51027908, 51027909, 51027910, 51027911, 51609601, 51609602, 51694101, 51694102

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I. Action Requested

Under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), OmniLytics, Inc. requests registration of an end-use product, AgriPhage-Nut & Stone Fruit (EPA File Symbol: 67986-RN), containing the new active ingredients bacteriophages active against *Pseudomonas syringae* pv.

syringae, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*. This active ingredient is formulated as a cocktail of four specific bacteriophage strains and is intended for use as a bactericide that is applied as a foliar spray (ground) and chemigation on nuts and stone fruit crops. Because application of the active ingredient may result in residues on food, OmniLytics, Inc. also requests establishment of a tolerance exemption for these four bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* in or on all food commodities. In support of registration, the applicant has submitted a Confidential Statement of Formula (CSF) (dated August 5, 2022), data matrix (dated July 6, 2022), product analysis data (MRIDs 51027901, 51027902, 51027903, 51027904, 51027905, 51027906, 51027907, 51027908, 51027909, 51694101, 51694102), mammalian toxicology data (MRID 51027910), and a tolerance exemption petition (1F8907, MRIDs 51027912 and 51609602).

II. Executive Summary

AgriPhage-Nut & Stone Fruit (EPA File Symbol: 67986-RN) is an end-use product containing 0.000025% bacteriophages active against *Xanthomonas arboricola* pv. *pruni*, 0.000025% bacteriophages active against *Xanthomonas arboricola* pv. *juglandis*, 0.000025% bacteriophages active against *Xanthomonas arboricola* pv. *corylina*, and 0.000025% bacteriophages active against *Pseudomonas syringae* pv. syringae, new active ingredients (0.0001% bacteriophages total). Bacteriophages are naturally occurring throughout the environment where host bacteria readily grow or infect. The specific phage strains contained in AgriPhage-Nut & Stone Fruit are isolated from naturally occuring sites of bacterial spot, canker, and blast on nuts and stone fruit trees. As a pre-harvest bactericide on food and non-food crops, its putative mode of action is to attach to target bacteria, lyse, and destroy the cells, thus acting as a pesticide against these disease-causing microorganisms.

Bacteriophages active against *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* have no demonstrated infectivity to humans and low acute toxicity, based on rationale citing scientific literature and additional information presented for the active ingredient and its closely related species. Dietary and drinking water exposure is expected to be negligible since significant residues are not expected due to the fact that bacteriophages persist only in the presence of the specific bacterial hosts (in this instance, *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*) and due to the self-limiting nature of viruses which die when dessicated, exposed to UV light, and heat. Additionally, no risk to humans is expected due to the highly specific nature of bacteriophages and the confirmed use of only lytic phages in this product.

There is potential for occupational exposure; however, no toxicological endpoints have been identified due to the self-limiting and highly specific host range of bacteriophages. There are residential uses proposed for the active ingredient but due to the expected low risk of these bacteriophages these uses are not likely to result in risks when product label instructions and precautions are followed. The Agency has determined that no further studies are needed at this time, considering all the available hazard and exposure data on bacteriophages active against *Pseudomonas syringae* pv. *syringae*, *Xanthomonas*

arboricola pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*. FIFRA Determination: Based on the available toxicology and exposure information, no unreasonable adverse effects to humans are expected from the use of bacteriophages active against *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* as a pesticide when EPA-approved product label instructions are followed. FFDCA Determination: Further, there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of bacteriophages active against *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* resulting from the proposed pesticidal uses.

III. Background

Bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* are naturally occurring microorganisms that have been discovered to attach to *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* bacteria, lyse, and destroy the cells thus acting as a pesticide against these disease-causing microorganisms. Bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* are ubiquitous and most commonly isolated from actual infected areas (bacterial spot, canker, and blast on nuts and stone fruit), naturally existing in each geographic region affected by *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*. Bacteriophage exposure occurs naturally and can be found in the human diet via phages present on the surface of a variety of plant-based foods and throughout the environment. There are currently no other pesticidal approvals or registrations for use of these microorganisms, however, similar bacteriophage-containing products have been previously approved for use on food crops (EPA Reg. Nos. 67986-1, 67986-6, 67986-8, 67986-9).

Bacteriophage population levels are expected to decrease to environmental background levels relatively rapidly following application due to depletion of target host bacteria and environmental factors (Balogh, 2010; Buttimer *et al.*, 2017, Chan, 2013; Jones *et al.*, 2012; Salmond, 2015). Bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*, therefore, will not likely result in significant residues on food or in water. According to the rationale submitted in support of toxicity/infectivity and acute toxicity study waivers, bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* are expected to have a low toxicity profile and no toxicological endpoints were identified.

IV. Product Identity and Analysis Review

The applicant summitted data to comply with product identity and analysis data requirements published in 40 CFR § 158.2120 in support of the registration of bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and

Xanthomonas arboricola pv. *pruni* and the end-use product AgriPhage-Nut & Stone Fruit. Table A1 (see *Appendix A*. *Product Identity and Analysis Review*) provides the status of the data requirements as published in 40 CFR § 158.2120 for bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* and the associated pesticide product for human health risk assessment. The data provided for the analysis of microbial identity and taxonomic classification of bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* and attribution to a wild-type, non-genetically modified lineage and has been cataloged within an internal database by the registrant, OmniLytics, Inc., with Agency access as necessary and consistent with prior similar registrations (US EPA, 2011). Additional genetic analyses are performed on these active ingredients which include full genomic sequencing and confirmation of absence of genes of concern (integrase, transposase, toxins) consistent with current scientific literature and technological capabilities. The nominal concentration of bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* contains no less than 1.25×10^9 PFU/mL for each phage strain within in the formulated end-use product. The technical grade of the active ingredient is used in the formulation of the final product in a direct process with no manufacturing use intermediate. The product identity, manufacturing process, discussion of formation of unintentional ingredients, analysis of samples, certification of limits, storage stability/corrosion characteristics, and physical/chemical characteristics data submitted for these products are acceptable.

V. Summary of Toxicology Data

Table 1 provides the status of the data requirements as published in 40 CFR § 158.2140 for bacteriophages of *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni* and the associated pesticide products for human health risk assessment. Requests to waive the generic (TGAI, technical grade of the active ingredient) data requirements for acute toxicity/pathogenicity and product-specific (EP, end-use product) toxicology data requirements were satisfied with justification and citation of publicly available literature and regulatory documentation. Information from the scientific rationales and cited studies are included in the section below. Additionally, quality control procedures outlined in the manufacturing process and analysis of samples ensure that each iteration of bacteriophages which are included in this and future formulations of AgriPhage-Nut & Stone Fruit have subjected to full genome sequencing for assessment of taxonomic classification and lack of integrase, transposase, and toxin genes and subject to lambda integrase, host specificity, generalized transduction, and turbid plaque assays. Therefore, it is reasonable to conclude that any bacteriophages which pass this screening are non-pathogenic and capacity to infect organisms aside from their target hosts or induce toxic effects.

The information provided is sufficient to satisfy the Tier I toxicology data requirements for human health risk assessment for the active ingredient and the associated pesticide products. Further testing at higher tiers is not required for the current label uses.

Table 1. Summary of data submitted to comply with toxicology data requirements published in 40 CFR § 158.2140 for support of the registration of products containing bacteriophages of *Pseudomonas*

syringae pv. *syringae*, *Xanthomonas arboricola* pv. *corylina*, *Xanthomonas arboricola* pv. *juglandis*, and *Xanthomonas arboricola* pv. *pruni*.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary, Classification and Toxicity Category (As Applicable)	MRID
Generic (TGAI) Toxicology Data			
Acute Oral Toxicity/Pathogenicity	885.3050	The rationale and supporting literature in MRID No. 51027910 support the claim that lytic bacteriophages are safe. There is no evidence of toxicity or pathogenicity associated with oral administration of bacteriophages. Bacteriophages are obligate intracellular pathogens of bacterial and not infectious in mammalian cells. This is consistent with rationale previously submitted to the Agency for similar bacteriophage active ingredients/similar products and deemed adequate to support registration in lieu of a formal guideline study. This formulation does not contain inert ingredients that are expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)). **Classification: Acceptable**	51027910
Acute Pulmonary Toxicity/Pathogenicity	885.3150	The rationale and supporting literature in MRID No. 51027910 support the claim that lytic bacteriophages are safe. There is no evidence of toxicity or pathogenicity associated with respiratory administration of bacteriophages. Bacteriophages are obligate intracellular pathogens of bacterial and not infectious in mammalian cells. This is consistent with rationale previously submitted to the Agency for similar bacteriophage active ingredients/similar products and deemed adequate to support registration in lieu of a formal guideline study. This formulation does not contain inert ingredients that are expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)). **Classification: Acceptable**	51027910
Acute Injection Toxicity/Pathogenicity	885.3200	This data requirement is not applicable to bacteriophages based on the test note 2 from 40 CFR 158.2140(d)(2), which states, "Data not required for products whose active ingredient is a virus." **Classification: Acceptable**	51027910
Hypersensitivity Incidents	885.3400	The applicant reported that no hypersensitivity incidents, including immediate-type or delayed-type reactions of humans and domestic animals, occurred during research, development, or testing of the TGAI over 7 years. Any future hypersensitivity incidents must be reported to the EPA (refer to test note #3 of 40 CFR § 158.2140(d)). **Classification: Acceptable**	51027910