

Guidance for Efficacy Testing of Antimicrobial Products Against Planktonic *Legionella pneumophila* in Cooling Tower Water

(8/28/2024)

Legionella pneumophila (*L. pneumophila*) is an aerobic, non-spore-forming, gram-negative bacterium of the genus *Legionella*. It is commonly identified as the causative agent of Legionnaires' disease, which is a disease acquired by breathing in water droplets contaminated with *Legionella* bacteria. Cooling towers are a potential breeding ground for this bacterium. Aerosolization of *L. pneumophila* can occur if cooling towers are not properly maintained. Outbreaks of Legionnaires' disease due to contaminated cooling tower water continue to occur.¹ Health departments reported nearly 10,000 cases of Legionnaires' disease in the United States in 2018.² However, Legionnaires' disease is thought to be underdiagnosed, thus this number likely underestimates the true incidence. A recent study by the Centers for Disease Control and Prevention (CDC) estimated that the true number of Legionnaires' disease cases may be 1.8 – 2.7 times higher than what is reported.³ The cost per hospital stay due to Legionnaires' Disease in 2012 – 2013 (in 2014 US dollars) has been estimated to be \$37,100 (\$7,950 – \$149,000).³

The United States Environmental Protection Agency ("EPA" or the "Agency") recognizes the public health implications of *L. pneumophila* and the importance of having antimicrobial products with demonstrated efficacy against this pest microorganism. Under EPA regulations, an antimicrobial pesticide is considered to make a public health claim if it "bears a claim to control pest microorganisms that pose a threat to human health, and whose presence cannot readily be observed by the user, including but not limited to, microorganisms infectious to man in any area of the inanimate environment."⁴ An applicant for a product bearing a public health claim must submit efficacy data to EPA with any application for registration or amended registration to support the claim.⁵ The Agency considers claims to reduce planktonic *L. pneumophila* to be public health claims because this microorganism is associated with Legionnaires' disease and poses a risk to public health. As such, EPA's regulations require the submission and review of appropriate efficacy data to support a determination that the product will work as expected.

¹ <https://www.cdc.gov/legionella/outbreaks.html>

² <https://www.cdc.gov/legionella/health-depts/surv-reporting/2018-19-surv-report-508.pdf>.

³ Collier SA, Deng L, Adam EA, et al. Estimate of burden and direct healthcare cost of infectious waterborne disease in the United States. *Emerg Infect Dis*. 2021;27(1):140–9.

⁴ 40 C.F.R. § 158.2204(a) (including additional factors for determining whether an antimicrobial product is making a public health claim).

⁵ 40 C.F.R. § 158.2220(a)(2).

This guidance provides the Agency’s recommended test method for evaluating the efficacy of antimicrobial pesticide products against planktonic *L. pneumophila* in cooling tower water, as well as sample pesticidal label use directions and claims to aid registrants in drafting clear use directions and claims for proposed products. Throughout this document, any use of the word **water** refers specifically to water used in cooling towers to remove heat from a process or environment.

This guidance is not binding on EPA or any outside parties, and EPA may depart from the guidance where circumstances warrant and without prior notice. Applicants may propose and submit alternative practices (e.g., modifications to the recommended test methodology) to the Agency for review. The Agency will evaluate any proposed method modifications for appropriateness on a case-by-case basis. This guidance may be updated in the future.

This document describes:

- Definitions
- Products intended to be covered by this guidance
- Test method for developing efficacy data supporting planktonic *L. pneumophila* claims
- Test criteria
- Data submission procedures for efficacy data
- Product use and labeling guidance

Definitions

This section defines certain terms for the purpose of this guidance.

Cooling tower: A cooling tower is a component of the larger cooling water system and serves as a specialized heat exchanger that removes heat from water mainly by means of latent heat loss from evaporation while coming into contact with an airstream. A cooling tower system may be comprised of an individual unit or multiple units.

Planktonic bacteria: Bacteria that drift, float, or swim weakly in a body of water. Does not include adherent or sessile bacteria that are attached to a surface (e.g., a biofilm).

Water management plan⁶: A plan describing a process to identify hazardous conditions and the steps to take to minimize the growth and transmission of *Legionella* and other waterborne pathogens in cooling tower water. A water management plan may include a plan that is

⁶ <https://www.cdc.gov/legionella/wmp/overview.html>

consistent with American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) guidelines or a plan that consists of an alternative cooling tower water system management strategy.

Remediation plan: A component of the water management plan that addresses cooling tower system issues and that requires taking short-term action(s) to bring the system water quality parameters, such as bacterial levels, total dissolved solids (TDS), water hardness, pH, etc., to acceptable levels as defined in the water management plan (and/or applicable federal, state/local regulation(s)).

Routine maintenance plan: A component of the water management plan that addresses the day-to-day operations of a cooling tower system to maintain the system's goals as defined in the water management plan (and/or applicable federal, state/local regulation(s)).

Products Intended to be Covered by This Guidance

This guidance provides a recommended test method that is most appropriate for evaluating the efficacy of liquid and solid water-soluble formulations. As a result, this guidance will be most useful for applicants seeking to add planktonic *L. pneumophila* claims to liquid and solid water-soluble formulations of an antimicrobial product. Please consult with EPA for other product formulation types.

Recommended Test Method for Developing Efficacy Data to Support Planktonic *L. pneumophila* Claims

The recommended test method and guidance provide a framework for registrants seeking to make a claim for certain antimicrobial products to reduce planktonic *L. pneumophila* in cooling tower water. Environmental and operating conditions in cooling towers can vary due to inherent water quality parameters and cooling tower system materials and design. Many of these conditions could impact a product's efficacy testing results. Due to this challenge, the method provides a set of testing conditions intended to simulate interferences representative of those observed in cooling tower systems.

EPA worked with stakeholders to determine what chemicals and concentrations are commonly used/found in cooling tower water. The testing solution includes yeast extract, which is a protein; 2-Phosphonobutane-1,2,4-Tricarboxylic Acid (PBTC), which is a scale inhibitor; Tolytriazole sodium salt (TT), which is a corrosion inhibitor; polyacrylic acid (PAA), which is a dispersant; and humic acid sodium salt, which is organic matter. EPA believes that the testing solution used in the method simulates interferences that are representative of cooling tower water under normal operating conditions.

To develop efficacy data to support planktonic *L. pneumophila* reduction claims pursuant to this guidance, EPA recommends the use of the latest version of EPA Microbiology Laboratory Branch (MLB) Standard Operating Procedure (SOP) [MB-42-00: Method for Testing Antimicrobial Products against Legionella pneumophila in Simulated Cooling Tower Water \(LSCTW\)](#).

Test Criteria

To support planktonic *L. pneumophila* reduction claims pursuant to this guidance, applicants should apply the following test criteria when evaluating products against planktonic *L. pneumophila*:

Acceptable Test Strain: Use *L. pneumophila* subsp. *pneumophila* (ATCC 33152).

Number of Batches and Test Flasks per Batch: Test three batches of the product, one batch per day, using separate test cultures, at the lower certified limit(s) (LCL) for the active ingredient(s) listed on the confidential statement of formula of the product. For each batch, evaluate three test flasks against the product, one untreated control flask, and one sterility flask.

Neutralizer Confirmation: Conduct neutralization testing to confirm and document the effectiveness of the neutralizer proposed for use in the efficacy test. Refer to the latest version of EPA MLB SOP MB-42. Refer to EN Standard 13623 Annex B for examples of neutralizers.

Product Diluent: For testing products that require dilution, use a diluent that is consistent with the Product Performance Test Guideline [OCSPP 810.2000 "General Considerations for Testing Public Health Antimicrobial Pesticides, Guide for Efficacy Testing"](#).

Interferents: For all tests, add 0.05% Yeast Extract, 450 ppm 2-Phosphononobutane-1,2,4-Tricarboxylic Acid (PBTC) (Scale Inhibitor), 75 ppm Tolytriazole (TT) sodium salt (Corrosion Inhibitor), 150 ppm Polyacrylic Acid (PAA) (Dispersant), and 0.5 g/L Humic Acid Sodium Salt to the test solution as specified in the latest version of EPA MLB SOP MB-42.

Control Counts: Each of the three aliquots taken from the untreated control flask should exhibit *L. pneumophila* counts of a minimum of 1.0×10^6 to a maximum of 1.0×10^7 colony forming units (CFUs) per milliliter.

Test Temperature: The test should be conducted at $30 \pm 1^\circ\text{C}$.

Performance standard for remediation treatment claims: Each independent test (conducted on separate test days) should demonstrate a minimum mean 5.0 log reduction (99.999%) in viable *L. pneumophila*.

Performance standard for routine maintenance treatment claims: Each independent test (conducted on separate test days) should demonstrate a minimum mean 3.0 log reduction (99.9%) in viable *L. pneumophila*.

Pursuant to this guidance, the claims above can be added to products individually if the submitted efficacy data and the label claims are consistent.

For oxidative chemistries: If more than a 100X starting concentration (T_0) of antimicrobial product is needed to achieve the desired active ingredient(s) [oxidant] level, that is within $\pm 10\%$ of the LCL, the applicant should validate the need for this additional chemistry in advance of or concurrently with efficacy testing. This information should be included in the final test report. Test three batches of product at the LCL listed on the confidential statement of formula of the product. For each batch, assess three samples to determine the mean concentration at T_0 ; these data should be recorded and included in the efficacy report. The resulting mean concentration for each batch should be within $\pm 10\%$ of the active ingredient(s) [oxidant] target LCL at T_0 .

The following criteria should be followed to determine if repeat testing is appropriate based on the mean active ingredient(s) concentration(s) at T_0 and corresponding efficacy results:

If the measured **mean** concentration at T_0 is **10%** of the **target** T_0 concentration and efficacy is demonstrated, results are acceptable.

If the measured **mean** concentration at T_0 is **less than 10%** of the **target** T_0 concentration and efficacy is not demonstrated, results are invalid and may be repeated per the repeat testing guidance discussed in the [810 FAQs](#).

If the measured **mean** concentration at T_0 is **greater than 10%** of the **target** T_0 concentration and efficacy is demonstrated, results are invalid and may be repeated per the repeat testing guidance discussed in the 810 FAQs.

If the measured **mean** concentration at T_0 is **greater than 10%** of the **target** T_0 concentration and efficacy is not demonstrated, results are considered valid; no repeat testing should be performed.

A new study may be conducted using 3 batches on independent test days by increasing the concentration and/or contact time.

Registrants should include the corresponding calculations with the efficacy data submitted for Agency review that demonstrate that the target testing concentration (T_0) is consistent with the free residual oxidant listed in the product's use directions (e.g., on the product label).

Modifications to the Test Procedures: When Agency-recommended methods are modified by the applicant to support specific claims for a product, the applicant should submit the complete testing protocol, identifying and describing each modification as well as a justification for the modification(s), for Agency review prior to initiating testing. All materials and procedures employed in the testing should be fully described.

Data Submission Procedures for Efficacy Data

To assist in the proper review and evaluation of product performance, submit complete descriptions of the test employed and the results obtained to the Agency following the [data submission guidelines](#). All product performance data must be developed in compliance with the [Good Laboratory Practice Standards](#) (40 CFR Part 160).

Product Use and Labeling Guidance

Antimicrobial products bearing claims for reduction of planktonic *L. pneumophila* added pursuant to this guidance may be used as part of a *L. pneumophila* water management plan for cooling tower systems. Owners and operators of cooling tower systems should develop and implement a water management plan that provides a multi-barrier approach to reduce *L. pneumophila* in cooling tower water, including routine maintenance and remediation treatments as recommended by the most recent American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) standards⁷ and/or applicable federal, state and/or local regulation(s). Please visit the [CDC website](#)⁸ for additional information to assist in the implementation of the ASHRAE standard(s). Ensuring that these complex cooling towers are operating in accordance with a water management plan will help maintain cooling tower system conditions that are appropriate for chemical remediation and maintenance.

⁷ANSI/ASHRAE. 2021. Standard 188-2021 – Legionellosis: Risk Management for Building Water Systems
ASHRAE. 2020. Guideline 12-2020 – Managing the Risk of Legionellosis Associated with Building Water Systems.
<https://www.ashrae.org/technical-resources/standards-and-guidelines/read-only-versions-of-ashrae-standards>

⁸ <https://www.cdc.gov/legionella/wmp/index.html>
<https://www.cdc.gov/legionella/wmp/toolkit/index.html>
<https://www.cdc.gov/legionella/downloads/Control-Toolkit-Cooling-Towers.pdf>

Exposure to *L. pneumophila* has been linked to Legionnaires' disease. Registrants may want to consider adding language to pesticide labels (and/or water management plan) that warns the pesticide user that when working in areas in which *L. pneumophila* may be present, one may want to consider wearing personal protective equipment (PPE) as recommended by the [Occupational Safety and Health Administration \(OSHA\)](#).

Refer to **Appendix** for sample "Directions for Use".

Pursuant to this guidance, EPA may approve the following "routine maintenance" and "remediation" claims, respectively, against planktonic *L. pneumophila* for use in cooling tower water for a product that successfully passes the latest version of EPA MLB SOP MB-42:

When used as part of an overall water management plan to manage cooling tower system conditions that may cause fouling in cooling tower water, this product can help reduce planktonic *L. pneumophila* bacteria in cooling tower water under normal operating conditions.

An antimicrobial product may be used as part of a water management plan to help reduce levels of planktonic *L. pneumophila* that exceed acceptable levels, as established in the water management plan and/or by applicable federal, state and/or local regulation(s). The water management plan may include other actions besides the use of an antimicrobial product (e.g., draining and/or cleaning the cooling tower, adjusting other water quality parameters) for reducing planktonic *L. pneumophila* levels in cooling tower water.

The registrant should tailor the efficacy testing protocol to the desired label claim(s) (e.g., chemistry compatibility with the product and/or the efficacy test). In addition, products intended for use against *L. pneumophila* in cooling tower water systems are required to meet all the labeling requirements of 40 C.F.R. § 156.10.

Examples of Label Claims:

- For **remediation** treatment, *in vitro* laboratory testing conducted using a(n) [X]-minute(s) contact time demonstrated a 99.999% (5-log₁₀) reduction of planktonic *L. pneumophila*.
- For **routine maintenance** treatment, *in vitro* laboratory testing conducted using a(n) [X]-minute(s) contact time demonstrated a 99.9% (3-log₁₀) reduction of planktonic *L. pneumophila*.

Appendix

Each label would contain Section 1 and Section 2a or Section 2b in the Directions for Use, as appropriate.

Section 1:

Legionella pneumophila Reduction in Cooling Tower Water Systems:

Water Management Plan: For use in cooling tower [systems] that have a water management plan consistent with the most recent ASHRAE standard(s) and/or applicable federal, state/local regulation(s). The water management plan should include guidelines for application of antimicrobial products that allow for the use of this product consistent with its directions for use. The water management plan should also define parameters for when routine maintenance and/or remediation treatment is applied. Please visit the CDC website for additional information to assist in the implementation of the ASHRAE standard(s). Ensuring that these complex cooling towers are operating in accordance with a water management plan will help maintain cooling tower system conditions that are appropriate for chemical remediation and maintenance.

A concentration of [X] ppm [free residual oxidant or active ingredient(s)] has been shown in laboratory testing to reduce suspended planktonic *L. pneumophila* subsp. *pneumophila* (ATCC 33152) within [contact time] for routine maintenance.

[AND/OR]

A concentration of [Y] ppm [free residual oxidant or active ingredient(s)] has been shown in laboratory testing to reduce suspended planktonic *L. pneumophila* subsp. *pneumophila* (ATCC 33152) within [contact time] for remediation treatment.

The efficacy evaluation method for this product is limited to planktonic *L. pneumophila* bacteria.

Section 2a: Example of Use Directions for an Oxidizing Product

INDUSTRIAL RECIRCULATING WATER COOLING TOWERS

Use according to the product label. For reduction of planktonic *L. pneumophila*, add appropriate amount of [PRODUCT] to obtain a free residual⁹ [oxidant] concentration of the product between [X to Y] ppm¹⁰, to the tower basin, distribution box, or some other point to ensure uniform mixing as described in the water management plan.

REMEDIATION

Before beginning remediation treatment, [drain and] prepare the cooling tower system as described in the water management plan.

Single Dose/Shock Treatment: Add appropriate amount of product to the system to obtain a free residual [oxidant] concentration of [X to Y] ppm. Apply product as described in the water management plan to reduce the levels of planktonic *L. pneumophila*.

Continuous Feed Treatment: Add and maintain a free residual [oxidant] concentration of the product between [X to Y] ppm. Monitor and maintain the product concentration as described in the water management plan to reduce the levels of planktonic *L. pneumophila*.

Intermittent Feed Treatment: Add and maintain a free residual [oxidant] concentration of the product between [X to Y] ppm. Monitor and maintain the product concentration as described in the water management plan to reduce the levels of planktonic *L. pneumophila*.

A free residual concentration of [X] ppm oxidant has been shown in laboratory testing to reduce the levels of planktonic *L. pneumophila* bacteria within [insert contact time] following single dose/shock treatment, continuous feed treatment, or intermittent dosage for remediation. This is the minimum time required to provide the log reduction for remediation. Ensuring that these complex cooling towers are operating in accordance with a water management plan will help maintain cooling tower system conditions that are appropriate for chemical remediation and maintenance.

⁹ The term “residual” refers to the amount of unreacted antimicrobial product available in the treated cooling tower water to reduce the number of bacteria and is measured as the concentration of free available antimicrobial product [oxidant].

¹⁰ Through sections 2a and 2b of this guidance document, the lower end of the range (X) is defined by the concentration used in efficacy testing, and the higher end of the range (Y) represents a target concentration that may be defined by registrants to account for variability in use.

ROUTINE MAINTENANCE

Continuous Feed Treatment: Add and maintain free residual [oxidant] concentrations of the product between [X to Y] ppm. Monitor and maintain the product concentration as described in the water management plan for planktonic *L. pneumophila* reduction.

Intermittent Feed Treatment: Add and maintain a free residual [oxidant] concentration of the product between [X to Y] ppm. Monitor and maintain the product concentration as described in the water management plan to reduce the levels of planktonic *L. pneumophila*.

A free residual concentration of [X] ppm oxidant has been shown in laboratory testing to reduce planktonic *L. pneumophila* bacteria within [insert contact time] following continuous feed or intermittent dosage for routine maintenance. This is the minimum time required to provide the log reduction for routine maintenance. Ensuring that these complex cooling towers are operating in accordance with a water management plan will help maintain cooling tower system conditions that are appropriate for chemical remediation and maintenance.

Section 2b: Example of Use Directions for a Non-Oxidizing Product

INDUSTRIAL RECIRCULATING WATER COOLING TOWERS

Use according to the product label. For reduction of *Legionella pneumophila*, add appropriate amount of [PRODUCT] per [XX] gallons of water to the tower basin, distribution box, or some other point to ensure uniform mixing as described in the water management plan. Monitor and maintain the concentration as described in the water management plan. Repeat until *Legionella* levels are reduced to those described in the water management plan.

REMEDIATION (Single Dose/Shock Treatment, Continuous Feed Treatment, or Intermittent Feed Treatment): Before beginning remediation treatment, [drain and] prepare the cooling tower system as described in the water management plan.

Add [X to Y] pound(s) or [X to Y] fluid ounce(s) of [PRODUCT] per [XX] gallon(s) of water in the cooling tower system to achieve a concentration of [X to Y] ppm of active ingredient(s). Monitor and maintain the product concentration as described in the water management plan.

A concentration of [X] ppm has been shown in laboratory testing to reduce planktonic *L. pneumophila* bacteria within [insert contact time] following remediation treatment. This is the minimum time required to provide the log reduction for remediation. Ensuring that these complex cooling towers are operating in accordance with a water management plan will help maintain cooling tower system conditions that are appropriate for chemical remediation and maintenance.

ROUTINE MAINTENANCE

Continuous Feed Treatment: Add [X to Y] pound(s) or [X to Y] fluid ounce(s) of [PRODUCT] per [XX] gallon(s) of water in the cooling tower system to achieve a concentration of [X to Y] ppm of active ingredient(s). Monitor and maintain the concentration as described in the water management plan.

Intermittent Feed Treatment: Add [X to Y] pound(s) or [X to Y] fluid ounce(s) of [PRODUCT] per [XX] gallon(s) of water in the cooling tower system to achieve a concentration of [X to Y] ppm of active ingredient(s). Monitor and maintain the concentration as described in the water management plan.

A concentration of [X] ppm has been shown in laboratory testing to reduce planktonic *L. pneumophila* bacteria within [insert contact time] following continuous feed or intermittent dosage for routine maintenance treatment. This is the minimum time required to provide the log reduction for routine maintenance. Ensuring that these complex cooling towers are operating in accordance with a water management plan will help maintain cooling tower system conditions that are appropriate for chemical remediation and maintenance.