Response to Public Comments on the Draft Guidance and Method for Efficacy Testing of Antimicrobial Products Against Planktonic *Legionella pneumophila* in Cooling Tower Water Docket ID No.: EPA -HQ-OPP-2023-0430

(08/28/2024)

In October 2023, the EPA Office of Chemical Safety and Pollution Prevention announced the availability and sought public comments on the draft guidance and test method (88 FR 67749, October 2, 2023 (FRL-11382-01-OCSPP)). The Agency received 41 comments regarding clarifications and revisions to the draft guidance and test method. This document summarizes the comments and provides the EPA response to comments received.

Commenter	Comment No. Method (M) or Guidance (G)	Comment(s)	EPA Response
1. Virginia Polytechnic Institute and State University	1 (M)	I have found several deficiencies in protocols described in the documents that would impact measurement of survival of *Legionella pneumophila* exposed to antimicrobial products. Those deficiencies are: (1) Following growth of *L. pneumophila* cells in laboratory culture medium, the cells must be collected and washed in sterile drinking water and then incubated in sterile drinking water for 1 week at room temperature to "acclimated" the laboratory-medium grown cells. Experience has taught that the response of cells grown in laboratory medium to antimicrobials do not reflect the response of water-acclimated cells. Water-acclimated cells are significantly more disinfectant-resistant, then cells only grown in laboratory medium. As *L. pneumophila* cells in cooling towers are acclimated to water,	Although many research groups have reported the use of resting or acclimated *L. pneumophila* cells in their studies, there are currently no standardized methods to generate these cell populations at a high and reproducible level for antimicrobial efficacy testing. Laboratory grown cells, by definition, are not identical to their environmentally propagated counterparts. Replicating this acclimation in the laboratory setting would be challenging since environmental conditions and waters are chemically and microbially diverse and complex. To help address this, EPA's recommended suspension test method simulates the cooling tower water environment with the inclusion of several interferents that may impact both the efficacy of the antimicrobial product and

		measurements of antimicrobial susceptibility of laboratory medium-grown cells would greatly underestimate the survival of water-acclimated *L. pneumophila* cells.	thus, susceptibility and inactivation of the target organism. Use of these interferents ensures a consistent, relevant, and reproducible challenge to both the antimicrobial product and *L. pneumophila* in each of the three independent test batches as described in the draft method.
	2 (M)	(2) In the protocols of the documents it is proposed that 5 gm/L Humic acid should be employed as an interferent for measures of antimicrobial susceptibility. It should be pointed out that humic acids stimulate growth of opportunistic premise plumbing pathogens (OPPPs) of which *L. pneumophila* is a member. Thus, measurements of antimicrobial activity will be subject to a combination of antimicrobial and growth-stimulatory activities. Unless the stimulatory activities of Humic acids is not measured independently of antimicrobial activity, there exists no control for stimulation of growth in the presence of antimicrobial.	The Microbiology Laboratory Branch (MLB) performed preliminary tests that assessed the testing solution with and without humic acid. No stimulation of *Legionella* growth was observed in the sample with humic acid, i.e., the control samples from solutions with and without humic acid were comparable.
2. Joseph Falkinham	3 (G/M)	The document's protocol for testing antimicrobial agents does not consider the possibility that an antimicrobial agent will trigger *L. pneumophila* to enter the Viable but Nonculturable state (VBNC). The VBNC state is a common response of *L. pneumophila* to stress, including exposure to antimicrobial agents. *L. pneumophila* cells in the VBNC state do not	The current method specifies a minimum mean of 5.0 and 3.0 log reduction in viable *L. pneumophila* to support a remediation and routine maintenance treatment claim, respectively. The current method does not assess the VBNC status of biocide-treated *L. pneumophila*. Currently, there are no standardized methods to determine the VBNC

		form colonies on microbiological media but are still viable and can be "resuscitated" to colony formation. If one were to perform a test of an antimicrobial agent against *L. pneumophila* and did not consider VBNC, the data would be misleading. Specifically, if VBNC cells were not considered, the extent of killing would be overestimated and a potentially ineffective antimicrobial accepted as effective for controlling *L. pneumophila*.	status of *L. pneumophila* in cooling tower matrices or post-efficacy treatment. Additionally, the exact conditions that would enable consistent and complete VBNC resuscitation to form colonies are also unclear and not available as a standardized method. Thus, without those standardized methods to provide robust and reproducible results, lab testing to assess VBNC status to support a label claim about efficacy would be challenging.
3. Tiffiny Graven	4 (G)	**SUMMARY-** The harm lies in the potential inadequacy of the guidance to comprehensively manage microbial risks in cooling tower systems, leaving room for the persistence and proliferation of harmful microorganisms beyond planktonic *Legionella pneumophila.*	Efficacy testing conducted to add claims to an antimicrobial pesticide product(s) is microorganism specific. This guidance and method are specifically targeted at planktonic *Legionella* control. Applicants interested in pursuing claims for other microbes should consult with the agency prior to testing to determine the appropriate methodology for product performance testing.
	5 (G)	**Pros:** **Focused Approach:** The document provides a clear focus on reducing planktonic *Legionella pneumophila*, addressing a specific concern in cooling tower systems. **Efficacy Testing:** The inclusion of efficacy testing guidelines is crucial for ensuring the effectiveness of antimicrobial products.	Thank you for your comment.
	6 (G)	**HARM**	In 2015, New York State published an emergency regulation requiring registration

		Limited Scope: The draft neglects adherent or sessile bacteria and other microorganisms in cooling tower systems, potentially leaving gaps in overall water quality management. **Incomplete Protection:** Focusing solely on planktonic *Legionella pneumophila* may leave the cooling tower system vulnerable to other harmful microorganisms that adhere to surfaces (biofilm) or are not addressed in the guidance. **Systematic Gaps:** The exclusivity to *L. pneumophila* may create systematic gaps, as different microorganisms with varying resistance levels may exist in cooling tower water. This narrow focus might not adequately address the diverse microbial challenges. **REMOVE HARM** **Expanded Scope:** Broaden the scope of the guidance to encompass a wider range of microorganisms commonly found in cooling tower systems. This includes addressing adherent or sessile bacteria and other potential pathogens beyond *Legionella pneumophila*.	and routine maintenance of cooling towers against *Legionella*. The registrant community then approached EPA with the concern that the Agency had no existing guidance for efficacy testing to support registration of antimicrobial pesticides including claims against *Legionella* in cooling towers. Given that infections by *Legionella* spp. occur via inhalation of small water droplets, the Agency believes targeting planktonic *Legionella* in cooling tower water is an important step to address stakeholders' concerns regarding outbreaks of Legionnaires' Disease associated with cooling towers. To address this concern, EPA worked alongside other federal partners and external stakeholders on the development of a method and guidance for planktonic cells of *Legionella*. In addition, this method and associated guidance are intended to be used in conjunction with a water management plan that should consider other cooling tower parameters and operational conditions (e.g., cleaning, management of additional water quality parameters, etc.) that could impact biofilm-related issues.

			The Agency may provide guidance on supporting claims against biofilm treatment in cooling towers in the future; however, additional research is necessary to better understand the feasibility of claims against biofilm in the diverse and complex cooling tower environment. Efficacy testing conducted to add claims to an antimicrobial pesticide product is microorganism specific. Thus, this labeling guidance and method are specifically targeting planktonic *Legionella pneumophila* control. Applicants interested in pursuing claims for other microbes should consult with the Agency prior to initiating efficacy testing to determine the appropriate methodology for product performance testing against other pathogens beyond planktonic *Legionella pneumophila*.
	7 (G)	**HARM** **Biofilm Consideration:** Lack of guidance on biofilm control may hinder comprehensive water system hygiene, as biofilms can harbor various pathogens. **Risk of Outbreaks:** Neglecting adherent or sessile bacteria increases the risk of biofilm formation, which can serve as a breeding ground for various pathogens. This oversight might contribute to waterborne disease outbreaks.	Though EPA recognizes the importance that biofilms may play in the survival of Legionella, this method and associated guidance is limited to claims related to treatment of planktonic *Legionella*. Given that infections by *Legionella* spp. occur via inhalation of small water droplets, the Agency believes targeting planktonic *Legionella* in cooling tower water is an important step to address stakeholders' concerns regarding outbreaks

		Limited Long-Term Effectiveness: Ignoring biofilm control strategies can result in decreased long-term effectiveness of antimicrobial products, as biofilms provide a protective environment for microorganisms, allowing them to persist despite treatment efforts. **REMOVE HARM** **Inclusion of Biofilm Management:** Integrate guidelines for biofilm control within cooling tower systems. This could involve recommending specific antimicrobial agents or practices to prevent or eliminate biofilm formation, reducing the risk of microbial persistence.	of Legionnaires' Disease associated with cooling towers. To address this concern, EPA worked alongside other federal partners and external stakeholders on the development of a method and guidance for planktonic cells of *Legionella*. Treatment against biofilms would entail considering several additional parameters including control of other microorganisms (e.g., amoebas). The Agency may provide guidance on supporting claims against biofilm treatment in cooling towers in the future. Nevertheless, products with planktonic *Legionella* claims are intended to be used in conjunction with a water management plan that should address other cooling tower parameters and operational conditions (e.g., cleaning, additional water quality parameters management, etc.) that could potentially address biofilm growth.
	8 (G)	**REMOVE HARM** **Regular Review and Updates:** Establish a mechanism for periodic review and updates to the guidance to incorporate emerging research findings and evolving industry best practices. This ensures that the guidance remains current and effective over time.	The Agency considers and incorporates the best science and practices to continually improve. As such, the guidance document indicates that it may be updated in the future. EPA aims to create ongoing opportunities for dialogue and collaboration with stakeholders

		Stakeholder Collaboration: Encourage collaboration with industry stakeholders, public health experts, and researchers to gather diverse perspectives and insights. This collaborative approach can lead to more robust and comprehensive guidance. **Communication and Education:** Alongside guidance, emphasizing communication and education efforts can raise awareness among stakeholders about the importance of proper disinfection and maintenance practices in cooling tower systems.	and partners using different communication and engagement tools for this and all Agency actions.
	9 (G)	I commend the EPA's initiative while I do suggest considerations for a more holistic approach and alignment with existing state regulations. I urge the EPA to prioritize the development and enforcement of comprehensive guidelines for cooling tower water management. It's essential to move beyond half-measures and ensure that procedures are not only established but rigorously adhered to. Proactive, thoughtful policies can prevent avoidable issues, safeguard public health, and contribute to a more resilient and secure environmental infrastructure. Thank you for considering these perspectives, and I encourage the EPA's commitment to a comprehensive and proactive approach in addressing microbial risks in cooling tower systems.	Thank you for your comments. EPA encourages the use of water management plans, which may include the use of pesticidal products depending on the specific conditions and/or needs of a cooling tower system; however, setting comprehensive “guidelines for cooling tower water management” is outside of the scope of this guidance and OCSPP’s authority under FIFRA, which focuses on the registration of pesticide products.

		Addressing and keeping in mind the effects of natural presence, warm water temperatures, aerosolization risk, lack of regulatory framework, environmental changes, and system design issues.	
4. Anastasia Swearingen / Hannah Alleman for **American Chemistry Council's Center for Biocide Chemistries (CBC)**	10 (G/M)	CBC welcomes EPA's proposed guidance and method, which address an important public health concern, the prevention of Legionnaires' disease.	Thank you for your comment.
	11 (G/M)	Cooling tower operators in parts of the United States are required to use products with efficacy claims against Legionella to comply with state and local regulations. The availability of the guidance and method for efficacy claims against L. pneumophila in cooling towers will likely increase the availability of compliant antimicrobial pesticides, for use as part of a water treatment plan.	Thank you for your comment.
	12 (G)	The guidance document offers definitions of key terms. CBC suggests an update to the definition of cooling tower to more precisely describe cooling towers: 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 water	Thank you for your comment. The Agency has revised the definition taking your feedback into consideration.

		system may contain a single or multiple cooling tower units. Cooling water systems are used for HVAC and refrigeration, industrial processes such as manufacturing and energy production, or for cooling equipment.	
	13 (G)	CBC also suggests updating the definition of planktonic bacteria in the definitions section and footnote 9. We suggest the change from "that attach" to "attached" to clarify that planktonic excludes bacteria that is already attached to another surface. Planktonic bacteria have the ability to attach to surfaces and we suggest the following change to clarify the definition: Bacteria that drift, float, or swim weakly in a body of water. Does not include adherent or sessile bacteria attached to a surface (e.g., a biofilm).	Thank you for your comment. The Agency has revised the definition taking your feedback into consideration.
	14 (G)	CBC notes that the development of efficacy methods for products used in cooling towers is challenging—each cooling tower is unique and complex, with various microorganism challenges, chemistries used, and other characteristics. The development of water management plans for each cooling tower system is an important step to help ensure the safe operation of cooling towers, including the reduction and control of *L. pneumophilia*. CBC appreciates EPA's integration of	Thank you for your comment.

		recommendations for the use of antimicrobial products as part of a water management plan compliant with ASHRE and/or federal, state, and local regulations as a core part of the recommended label language.	
	15 (G)	CBC suggests removing soluble and concentrate from the types of products intended to be covered by the draft guidance. The method can be appropriately applied to additional products that are not soluble liquids or concentrates. In particular, not all solid products used are concentrate formulations.	Thank you for your comment. The Agency sought clarification from the commenter which was intended to revise the syntaxis to clarify the meaning/intent of the statement and to align the statements with how the chemistries are typically described and/or applied. We revised the language taking this comment into consideration by rephrasing "soluble liquid and solid concentrates" to "liquid and solid water-soluble products". We also removed the word "concentrates" as it may imply that the product is prepared to a use-dilution prior to application which is not typically the case for products applied to cooling towers.
	16 (G)	While the proposed label language from EPA is comprehensive, flexibility is needed in wording for different types of products. We note that some of the language could be redundant and may not be needed for all types of products. The guidance notes that the label language in the appendix is "example" and we seek clarification that the text provided in Section 1, 2a, and 2b is provided as an example that can	Yes, language in sections 2a and 2b is meant to be an example and is anticipated to be revised according to the product's specific application needs. Application of each product will also be informed by the cooling tower system's water management plan.

		be modified by registrants for product needs, with the appropriate EPA review and acceptance.	
	17 (G)	On the top of page 6, the Appendix is noted to contain sample "Directions for Use." However, not all language included in the Appendix is appropriate for the Directions for Use section of the label. Personal protective equipment (PPE or Personal Protection, as written in the Draft Guidance), is typically found in the Precautionary Statements section of the label, per the Label Review Manual. The language on waste disposal is typically included in the "Storage and Disposal" section of the label, which is typically found at the end of the Directions for Use section, clearly set apart as described in the EPA Label Review Manual Chapter 13. The language in the draft guidance on waste disposal is also not unique to a product with a *L. pneumophilia* efficacy claim. The way Appendix Section 1 is written, it seems to suggest these items should be grouped together and that these are unique requirements for products with *L. pneumophilia* claims. We suggest clarifying that language on PPE can go into the precautionary statements section and further that the PPE requirements for use in *L. pneumophilia* reduction may not differ from the PPE requirements for the products'	The guidance was revised by deleting the "Waste Disposal" bullet from the Appendix. The statement regarding PPE was revised and moved under the section "Product Use and Labeling Guidance" as follows: "Exposure to *L. pneumophila* has been linked to Legionnaire's 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)."

		non-public health use. We also suggest clarifying that the elements in Section 1 should be on the label, but not in a particular order.	
	18 (G)	In Appendix Section 1, the language surrounding water management plans could be shortened to remove the reference to "other water management strategies." CBC is unclear as to what other water management strategies would include.	This language was intended to provide flexibility with regards to the application of alternative management strategies that may not fit a "water management plan". Smaller systems may require simpler plans/approaches that may not necessarily strictly follow ASHRAE's guidelines. However, the Agency has revised the guidance language to simplify this message by revising the "Water management plan" definition.
	19 (G)	For the remediation directions in both section 2a and section 2b, we suggest changing the language from "Clean system" to "Prepare system" before beginning remediation treatment. This is because water management plans often describe steps beyond just cleaning a system before beginning remediation treatment. CBC appreciates the recognition that few systems can be fully drained before remediation treatment can begin, with the inclusion of "drain" in brackets.	Thank you for your comment. The Agency has revised the guidance taking your comment into consideration.
	20 (G)	**Under Section 2a: Example of Use Directions for [an] Oxidating Product, CBC suggests removing "per xx gallons of water."** When cooling tower operators are dosing with	Thank you for your comment. The Agency has revised the guidance taking this comment into consideration by deleting "per XX gallons of water".

		oxidative chemistries, they are dosing on demand until they hit the appropriate residual, as described in the label language. They do not measure doses per gallons of water, therefore including such dosage instructions on the label could be confusing or result in use not adhering to label directions. This dosing to the residual should be elaborated in each cooling tower's water management plan, as referenced on the label.	
	21 (G)	[…]the example label instructions have language, "treatment has been shown in laboratory testing to reduce suspended *L. pneumophila* subsp. pneumophila (ATCC 33152) within [contact time]." CBC suggests removing the reference to contact time in the example label language. CBC notes that the method does not specify a maximum contact time allowable to achieve the necessary log reduction for remediation or maintenance doses. Further, in field settings, cooling tower operators utilize their water treatment plans, including dosing to a residual, which may not lend to a particular contact time. Therefore, including contact time on the label may cause confusion for the operator.	Thank you for your comment. The Agency decided to keep the original guidance language as it is intended to state the contact time used during efficacy testing which shows **the minimum** contact time required to achieve the intended Log reduction under the laboratory testing conditions. However, the Agency recognizes that the contact time used during laboratory testing may not reflect the contact time used during "real life" field applications, and we expect that cooling tower managers/operators will refer to their water management plan when applying the product in field settings.
	22 (M)	CBC appreciates the considerable work from EPA and collaboration with registrants to develop the data used to refine the draft method for testing antimicrobial products against *Legionella pneumophila* in simulated	If the statement refers to a product that produced an LR = 4.9 in at least one of the 3 required tests, then because 4.9 is under the requirement of 5, the conclusion by EPA is that the data do not provide enough evidence

		cooling tower water. CBC notes that the results from this method show very high error rates, particularly with the possibility of false fails for effective products. Further statistical analysis could result in a greater understanding of the true extent of pass and fail error rates. For example, the definition of an ineffective product for remediation was set at a mean log reduction less than or equal to 4, when a product would be considered ineffective by EPA at 4.9 if tested under the Antimicrobial Performance Evaluation Program (APEP).	at a 95% confidence level that the product is effective (where an effective product is defined as having a mean LR of 4 or more in Table 2 from EPA-HQ-OPP-2023-0430-0007, page 66). Three test results with all tests yielding LR ≥4.9 still provide some evidence that the product is effective (i.e., has a mean LR >4); however, the associated confidence level is less than 95%. If the statement refers to a hypothetical product with a true mean LR of 4.9, then this product is by definition considered effective (because 4.9 is larger than 4); however, it is unlikely (less than 78% chance, see Table 2 from EPA-HQ-OPP-2023-0430-0007, page 66) that the product will provide enough evidence at 95% confidence in 3 tests that the product is effective (i.e., has a true mean LR > 4). In addition, “very high error rates” is a subjective phrase. If the product testing targets an LR of 6.0, the pass error rate is <5% with a pass error rate of 22%. However, if the product testing targets an LR of 6.35, the pass and fail error rates are <5%. Thus, creating a product that targets a higher LR will result in a lower chance that the product will inadvertently fail the efficacy test.

	23 (M)	While CBC is concerned with the recent trend in methods having high error rates, in this particular method for efficacy claims against *L. pneumophila* in cooling towers, we believe the high error rate is of less concern because there are not contact time requirements in the method. As the method is silent on contact time, CBC seeks confirmation that there are no contact time requirements.	Yes, we confirm that the method does not prescribe a specific contact time.
	24 (M)	*CBC also offers the following specific comments on various lines within the method:* **Header of each page:** Remove [Type here] due to typographical error.	Revised based on comment.
	25 (M)	**Line 151:** Per the guidance, this triggers the need for additional monitoring of AI levels. This should be addressed in the method. The means of confirming the active ingredient will vary for each active.	This is addressed in the guidance Appendix, Section 2a.
	26 (M)	**Line 198:** Please allow the use of alternatives for the PETG 250 mL disposable Erlenmeyer flasks with vented cap. Not all laboratories may have these available, which may limit the use of the method.	For biosafety reasons, it is recommended to use these materials with screw caps and vented closures. Language in the SOP was modified and made more generic.
	27 (M)	**Line 202:** Please allow the use of alternatives for the 50mL bioreaction tubes. Not all laboratories may have these available, which may limit the use of the method.	For biosafety reasons, it is recommended to use these materials with screw caps and vented closures. Language in the SOP was modified and made more generic.

	28 (M)	**Line 227:** Per Section IV(B)(1), a water bath is also needed for BCYE agar equilibration prior to adding the iron and cysteine.	Added the wording to line 227.
	29 (M)	**Lines 240-245:** We propose that the 72-hour BCYE plate check discussed in this section be included as optional rather than mandatory. This is because there is a culture purity control also performed on each test date, confirming further that no contaminants are present and the BCYE plate has pure growth from each day of testing. The 72-hour check of the initial BCYE plate poses unnecessary, additional work and documentation during a GLP test, as it is often difficult to remove and return plates to incubation in a GLP setting.	The method has been revised to make clear that the 72-hour check is optional.
	30 (M)	**Line 302 (starting section):** The example contact time has a +/- range here of 1 minute, but then there is a second statement that transfer into neutralizer must be within 30 seconds of the end of the contact time. These two sentences appear to conflict with one another. Does this mean that if you have a 60±1 min contact time, the transfer can happen within 30 sec after 61 minutes? Is ±1 min also acceptable for shorter contact times?	Thank you for the comment. We have changed the end of the contact time to be ± 5 seconds (regardless of the contact time), to align with other methods. The analyst will have 30 seconds to get all 3 samples transferred.
	31 (M)	**Line 308:** If the neutralizer volume is increased to 19 mL, does the neutralizer tube still represent the 10-1 dilution as stated above when using 9 mL neutralizer?	Yes, the tube still represents the 10^{-1} dilution, to account for larger neutralizer volume in calculations.

	32 (M)	**Line 353:** When TNTC (Too Numerous to Count) values are observed for each dilution filtered, please confirm if a symbol should be included in the calculations (i.e., ≥200 CFU) or if the value should be 200 CFU.	For calculation purposes, substitute 200 at the highest (most dilute) dilution.
	33 (M)	**Line 380:** Please clarify what "and scale up accordingly" means in terms of the calculations and provide an example calculation.	Use 0.5 CFU/mL as the number of CFU recovered from the 10^{-1} and 0 CFU for the remaining dilutions filtered.
	34 (M)	**Appendix 2 (bottom of page):** The "Draft Legionella Test Method v.08/23/23" text needs to be moved so that it does not overlap with the schematic.	Correction made.
	35 (M)	**Line 435:** As the neutralization control must achieve a narrow input of ≤200 CFU per 0.1 mL, please provide the flexibility to allow for the use of more than two serial dilutions, if desired by the performing laboratory.	Revised to reflect flexibility to assess more than 2 dilutions.
	36 (M)	**Neutralization Confirmation Assay Flow Chart (top of p. 25):** In previous drafts of the method, there is a statement under the set of three arrows under Treatment 1 that states "At timed intervals, transfer 1mL to each of 3 tubes containing 8.9 mL neutralizer and vortex mix. Hold for 30 seconds." CBC suggests adding this back into the method. Furthermore, descriptive text regarding the transfer of aliquots into neutralizer appears to be missing from this schematic. Please add this additional detail to the schematic for clarity.	Language revised. The additional detail is written in the schematic.

	37 (M)	**Line 441:** The 10 min±30 second hold period after addition of *L. pneumophila* should be at least as long as it takes to dilute and filter plate the samples in the testing. This 10 minute hold period may not be representative of how the test and control samples are handled during testing, so we request that the hold period be flexible to align with how testing is performed.	The purpose of the 10 min hold period is to assure adequate neutralization of test chemical; this hold-time is comparable with other methods quantitative neutralization methods.
	38 (M)	**Line 472:** Counts below 20 CFU/filter should still be considered valid if the control passes as a lower input (<20 CFU) would represent a more stringent neutralization control. CBC proposes a target range of 20-200 CFU/filter, but requests that the acceptance criteria be revised to ≤200 CFU/filter to allow for a passing control in the event that <20 CFU/filter are observed and the neutralization control passes.	The target counts of 20-200 CFU/filter are used to provide standardization to the neutralization procedure. Individual plate counts less than 20 may be acceptable provided that the mean counts for the treatment are ≥20.
5. Shannon Emerson for **Ecolab**	39 (G/M)	Ecolab appreciates the opportunity to review and provide comments on the Draft Guidance for Efficacy Testing of Antimicrobial Products Against Planktonic *Legionella pneumophila* in Cooling Tower Water and the corresponding efficacy test method, "Method for Testing Antimicrobial Products against *Legionella pneumophila* in Simulated Cooling Tower Water (LSCTW)". We thank the Agency for their hard work and dedication to this important public health concern.	Thank you for your comment.

	40 (G)	We encourage the Agency to reconsider their recommended approach to performing efficacy testing using oxidative chemistries dosed on residual in the draft guidance document. Due to the reactive nature of oxidant chemistries and their interactions with the added test system interferents meant to simulate a worst-case Cooling Tower system, it is likely that the target residual free oxidant levels will decrease substantially after initial flask dosing (T0). Targeting and maintaining the residual oxidant concentration within ±10% of the intended use levels may not be feasible over the course of the contact time(s) following initial test flask treatment and may result in considerable variability. Therefore, Ecolab recommends removing the requirement to maintain the target concentration over the contact time for oxidative chemistries.	We recognize the concern and challenges of maintaining a free oxidant residual within ±10% for the duration of the contact time. As a result, we have revised the guidance to reflect that the resulting mean concentration of the active ingredient for each batch tested should be within ±10% of the target LCL free residual [oxidant] **at T_0** rather than for the duration of the contact time.
	41 (G)	Additionally, oxidative chemistries dosed on residual cannot be prepared using the LCL from the CSF for efficacy testing. This is because the listed active ingredient on the CSF does not always directly correlate to the amount of residual free oxidant observed in the test system. Therefore, Ecolab recommends targeting the lowest nominal concentration of residual free oxidant listed in the use instructions for efficacy testing.	The Agency recognizes that the active ingredient concentration(s) listed on the CSF do not always directly correlate to the amount of free oxidant observed in the test system. However, theoretical calculations of expected free oxidant based on the product formulation and a ratio of the **LCL:Nominal concentration(s)** on the CSF may be used to calculate a representative target LCL concentration for testing oxidative chemistries at T_0. The registrant should include the corresponding calculations that

			demonstrate that the target testing concentration is consistent with the free residual oxidant listed in the use directions with the submitted efficacy data for Agency review.