



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

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Draft Guidance to Assess the Efficacy of Antimicrobial Pesticide Products Intended to Control Public Health Biofilms on Hard, Non-Porous Surfaces

This document provides the Environmental Protection Agency's (EPA or the agency) draft guidance for antimicrobial product claims for the control of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms on hard non-porous surfaces. The term "biofilm" is reserved for claims against biofilm that contain specific bacteria that are directly or indirectly infectious or pathogenic to humans. Thus, biofilm claims are considered to be public health claims for which the agency must review and approve appropriate efficacy data. Slimicide claims are considered to be non-public health claims as such slimes are of a purely economic or aesthetic significance and are not associated with claims for the control of pathogenic bacteria.

This document describes:

- Biofilms and their public health significance;
- Test procedures for developing efficacy data supporting biofilm claims;
- Products that may be eligible for biofilm claims;
- Test criteria;
- Data submission procedures for efficacy data; and
- Labeling guidance.

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

Biofilms

Bacteria may grow and persist in the environment as free floating (planktonic) cells in liquids or attached to surfaces as biofilms. Biofilms form when bacteria adhere to environmental surfaces, especially those located in the presence of high moisture. Biofilms typically persist in a matrix containing slimy, glue-like substances (extracellular polymeric substances) which facilitate their attachment to many hard surfaces such as glass, metals, and plastics, including those in health-care settings. The biofilm matrix provides embedded bacteria with protection from dehydration and other environmental stresses, and may interfere with the action of chemical disinfectants.

Thus, bacteria in biofilm have the capacity to survive environmental stress and may serve as a reservoir of human pathogenic bacteria.

The EPA considers claims to control pathogenic bacteria in biofilms to be public health claims for which the agency must receive, review, and approve appropriate efficacy data. Examples of use sites that may be supported by the biofilm test methodologies herein, and found acceptable, include restrooms, shower stalls, sink basins or drains (excluding the drain pipe) and nearby hard, non-porous surfaces of walls, countertops, and instrument trays in patient care areas of hospitals. In contrast, claims against non-public health slimicides must also be supported by appropriate efficacy data, however, submission of the data is only required when requested by the EPA.

Proposed Test Methodologies

The EPA recommends the use of two methods presented here as Standard Operating Procedures (SOP); EPA Microbiology Laboratory Branch (MLB) SOP MB-19, “Growing a Biofilm using the CDC Biofilm Reactor,” and EPA MLB SOP MB-20, “Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilms” to evaluate public health biofilm claims. The two methods are adapted from ASTM standard methods E2562-12 and E2871-13, respectively. SOP MB-19 is utilized to generate the *Pseudomonas aeruginosa* or *Staphylococcus aureus* biofilm on coupons; the coupons are removed from the reactor and evaluated using the procedure outlined in SOP MB-20 to determine the effectiveness of the antimicrobial product. The Single Tube Method is designed to evaluate the reduction of bacteria in biofilm for water soluble powders or liquid formulations.

Note: The proposed biofilm test methodologies are not suitable for use sites associated with water systems due to differences in nutritional status, flow rate, water treatment processes (e.g., chlorination), and other environmental conditions found in water systems (e.g., drinking water distribution systems, cooling tower systems). Therefore, the agency does not expect that the proposed biofilm test methods would accurately demonstrate the efficacy of antimicrobial products against public health biofilms in water systems. Proposed protocols for evaluating the efficacy of antimicrobial products against biofilms in water systems may be evaluated by the agency on a case-by-case basis.

Eligible Product Types

SOP MB-20 is designed to evaluate the reduction of bacteria in biofilm for water soluble powders or liquid formulations. Spray formulations may be evaluated as a liquid by removing/dispensing an appropriate amount of liquid from the spray device and following SOP MB-20 for the efficacy evaluation. The proposed method is not recommended to support public health biofilm claims on towelette products because the volume of liquid necessary to conduct the efficacy evaluation greatly exceeds that which can be easily expressed from towelettes. Applicants should consult with the agency for other formulation types prior to developing data in support of registration. The agency may require submissions of protocols for review and approval prior to commencement of testing.

If approved by the agency, biofilm claims may be added to products supported by acceptable disinfection efficacy data generated against both *Pseudomonas aeruginosa* and *Staphylococcus aureus* using appropriate test methods, such as those outlined in the most current version of the EPA's Product Performance Test Guidelines (OCSPP 810.2200 for liquid and spray formulations). As of the date of this guidance, the most current methods include the AOAC Use-Dilution Methods (2013) and the AOAC Germicidal Spray Products as Disinfectants Method (2013). In addition, the agency will assess the acceptability of the product formulation and labeling for a biofilm claim.

Test Criteria

Applicants should follow a two-step process to assess the efficacy of antimicrobial products, including hospital disinfectants, intended to control public health biofilms. First, *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms should be grown according to SOP MB-19: "Growing a Biofilm using the CDC Biofilm Reactor." Second, product efficacy data using coupons from the CDC reactor should be generated according to SOP MB-20: "Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm."

Acceptable Test Strains and Generation of Test Cultures: *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538). Stock cultures and a mature biofilm should be produced following SOP MB-19.

Number of Batches and Test Coupons per Batch: Test three batches of the product at the lower certified limit(s) (LCL) listed on the confidential statement of formula of the product against both test microbes for a total of six tests conducted on independent test days. Evaluate a minimum of five coupons against the product and three coupons as controls. Testing should be conducted at a single laboratory.

Neutralizer Confirmation: Neutralization testing should be conducted in advance of efficacy testing to confirm and document the neutralizer's effectiveness for the product. Refer to SOP MB-20, Attachment 1.

Contact Time: The contact time for testing should not exceed 10 minutes.

Diluent: The product's diluent should be consistent with that used for the evaluation of claims based on the most current version of the EPA's Product Performance Test Guidelines, OCSPP 810.2200 for liquid and spray formulations.

Control Counts: The mean log density for coupons (colony forming unit (CFU) per coupon) inoculated with *P. aeruginosa* or *S. aureus* should be 8.0-9.5 (corresponding to a geometric mean density of 1.0×10^8 to 3.2×10^9), based on data collected from multiple collaborative studies.

Product Performance Criteria: Attain a minimum 6 log reduction in viable bacteria in biofilm. Meet the performance standard in each of six individual tests.

Additional Bacteria: The agency will evaluate claims against additional bacteria in public health biofilms on a case-by-case basis, provided that the product meets the performance criteria for testing against *P. aeruginosa* and *S. aureus* using tests such as those outlined in the most current version of OCSPP 810.2200 for liquid and spray formulations, and the performance criteria for a public health biofilm claim for *P. aeruginosa* and *S. aureus*. Applicants should consult with the agency prior to developing an alternative biofilm growth protocol or efficacy data to support approval of such claims.

Modifications to the Test Procedures: When 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 to the agency for review and evaluation prior to the initiation of the tests. All materials and procedures employed in the testing should be fully described.

Data Submission

To assist in the proper review and evaluation of product performance, complete descriptions of the test employed and the results obtained should be submitted to the agency. All test reports should include, at the least, the information cited in the agency's Product Performance Test Guidelines, OCSPP 810.2000, General Considerations for Testing Antimicrobial Agents (refer to the section on data submission and reporting). The Good Laboratory Practice Standards (GLPs) regulations at 40 CFR 160 apply to studies to support registration of all antimicrobial products.

Labeling

Identification of Specific Use Sites for Public Health Biofilm Claims: Labeling needs to contain directions specific for treating hard, non-porous surfaces upon which public health biofilms are expected to form. Examples of use sites that may be acceptable include restrooms, shower stalls, sink basins or drains (excluding the drain pipe) and nearby hard, non-porous surfaces of walls, countertops, and instrument trays in patient care areas of hospitals.

The agency will evaluate additional use sites (other than hard, non-porous surfaces) on a case-by-case basis. Applicants should consult with the agency prior to developing efficacy related registration data for such use sites.

Examples of Acceptable Label Claims Against Public Health Biofilms:

- Kills 99.9999% of bacteria* in biofilm on a hard, non-porous surface.
- Kills a minimum of 99.9999% of bacteria* in biofilm
- Reduces at least 99.9999% of bacteria* growing in biofilm
- Formulated to kill 99.9999% of bacteria* in biofilm
- Other related claims:
 - Kills biofilm bacteria*
 - Penetrates biofilm, killing the bacteria* living there

*[List of bacteria "tested as a biofilm"; at a minimum, *Pseudomonas aeruginosa* and *Staphylococcus aureus*]

The agency will evaluate other label claims against biofilms on a case-by-case basis.

Special Label Instructions for Cleaning and Disinfecting Public Health Biofilms: All products bearing public health biofilm claims need to include these directions:

Cleaning and Disinfection Procedure for Hard, Non-Porous Surfaces: Pre-clean surfaces to remove soil and filth. Wipe dry. Thoroughly wet pre-cleaned surface with product. Allow surface to remain wet for [contact time]. Rinse thoroughly.

Acceptable Non-public Health Claims: Under 40 CFR 158.2220 (a)(3), each product that bears non-public health claims must be supported by appropriate efficacy data. The EPA reserves the right to require, on a case-by-case basis, submission of such data at any time. Product labeling for efficacy claims against non-public health slimes of economic or aesthetic significance may include labeling for “slime-forming bacteria,” “slime-forming microorganisms,” or “slime.” Several terms may be accepted on product labels with such non-public health claims, for example:

- Slimicide
- Cleans away microorganism slime/grunge
- Maintains control of slime
- Controls slime-forming microorganisms.