US Environmental Protection Agency
Office of Pesticide Programs

Questions on Copper Surface Efficacy Protocol

January 27, 2016

Protocol for the Evaluation of Bactericidal Activity of Hard, Non-porous Copper Containing Surface Products

Questions for the Letter Peer Review Panel

1. Suitability of the Protocol: The protocol has been revised to include additional methodology for preparing the test microbe, chemical and physical abrasion, details on materials and supplies, and quality control practices. Refer to Appendix A for an overview of the protocol changes. Please comment on the following issues:
    a. Do the revisions summarized in Appendix A provide a substantial improvement and technically sound approach for testing the antimicrobial properties and product durability of solid copper/copper alloy materials? If not, please provide advice on any additional elements that should be addressed or modified.
    b. Is the protocol of sufficient detail so that it may be conducted by a qualified testing laboratory and is likely to result in reproducible results when conducted in different testing facilities and/or at different times within the same laboratory? If the protocol is not of sufficient detail, which areas require improvement?
    c. Is the protocol suitable for evaluating the antimicrobial efficacy of solid copper/copper alloy materials, as well as copper-impregnated or coated surface materials? If the protocol is not suitable for one or both, please provide advice on how to modify the protocol to cover both materials.
    d. There is an interest in using the protocol to evaluate other types of hard non-porous surfaces impregnated with antimicrobial agents other than copper (other solid metals, metal alloys, fabricated materials etc.). Is the protocol suitable for testing the antimicrobial activity of other types of hard, non-porous surfaces treated or impregnated with antimicrobial agents? If not, please explain why and offer advice on how to change the protocol so that it would produce reliable, reproducible results when testing these other surface types.

2. Controls: Stainless steel was selected as the control carrier material due to the inert nature of the material. The final log reduction values are calculated by taking the $\log_{10}$ difference between the stainless steel control carriers and the product test carriers.
    a. In the protocol, the stainless steel control carriers are not subjected to the mechanical surface abrasion or the chemical treatments (A, B, and C). Please comment on the suitability of this approach. If this approach is not appropriate, please provide advice on how to address the management of the control carriers.
    b. Please comment on whether the comparative analyses of log reduction values (i.e., the difference in the level of microbes on exposed carriers vs. unexposed carriers and stainless steel control carriers) is a technically sound approach to the assessment of the antimicrobial activity of the copper and copper alloy products.

3. Contact Time: EPA's current guidance requires that a hospital disinfectant kill between five to six logs (100,000 to 1,000,000) of the target microbe in a qualitative test system within the time frame specified on the product labeling. The use of copper and copper alloy products in medical care facilities is a supplement to (not a replacement for) standard infection control practices and use of EPA registered hospital disinfectants.

a. In a standard chemical disinfectant test, the microbe is applied to the carrier surface, allowed to dry, and then exposed to the disinfectant. The contact time for the chemical disinfectant begins upon application of the disinfectant. For copper and copper alloy materials, the surface serves as the antimicrobial agent. The protocol specifies that the contact time begin upon application of the microbe to the surface, not after the microbe has dried on the surface. Please comment on whether it is appropriate for the contact time to begin upon inoculation of the surface, and if not, please offer alternative approaches for this step in the protocol.
b. Please comment on whether a single inoculation per carrier (4-5 logs bacteria per carrier) for both *Staphylococcus aureus* and *Pseudomonas aeruginosa* provides adequate challenge to evaluate the level of antimicrobial activity. A soil load (three-part) is also added to the inoculum before carrier inoculation. If a single inoculation is not appropriate, explain why and provide suggestions on how to improve the inoculation procedure.
c. Based on the Agency's experience in utilizing hard non-porous carriers in standard efficacy test methods, microbial populations on environmental surfaces decline naturally over time mainly due to desiccation. This natural decline presents challenges in determining whether the decline in a microbial population is due to desiccation or antimicrobial activity. An antimicrobial surface such as copper should be capable of accelerating the decrease in the number of surface-associated bacteria. The Agency expects that an antimicrobial effect due to the product should be measureable within a one hour timeframe. The original protocol specified a 99.9% reduction of viable bacteria within two hours of inoculation while the new protocol specifies a one hour timeframe. Please comment on the suitability of reducing the timeframe from two hours to one hour, or if the specified timeframe is not reasonable, provide advice on a suitable timeframe.
d. Please comment on whether copper and copper alloy products that kill 99.9% of target microbes within 1 hour would provide a significant benefit in reducing levels of target microbes in medical care facilities. If you think that killing 99.9% of target microbes within 1 hour would not provide a significant benefit in reducing levels of target microbes in medical care facilities, please offer advice on the level of antimicrobial activity that would provide such benefits. Please explain the basis for your conclusions.

4. <u>Abrasion/Chemical Treatment</u>: The proposed protocol includes a requirement that the carriers made from the copper or copper alloy undergo both an abrasion step and a chemical treatment step in order to simulate actual conditions of use and to evaluate how abrasion and/or chemical treatment might affect the level of antimicrobial activity. Note that some disinfectant and sanitizer products, as well as some cleaning agents, contain chelating agents (e.g., EDTA) intended to bind free metal ions.
    a. Please comment on whether abrasion and/or chemical treatment is likely to affect the level of antimicrobial activity displayed by a product. If not, please explain why. If so, please comment on how well the proposed abrasion step and chemical treatment step

reflect the likely range of actual use conditions. To the extent that the simulated conditions do not reflect the likely range of actual use conditions, please comment on whether the additional requested information (quantitative and qualitative) about the durability of the product is sufficient to assess the potential for physical disruption of the product surface after long term use.

b. If you think that abrasion and/or chemical treatment may affect antimicrobial efficacy, but that the proposed protocol does not adequately evaluate the potential for such effects, please offer advice on how to change the protocol (e.g., what process and/or chemical solutions should be used to treat a carrier) so that the protocol will adequately evaluate the level of antimicrobial activity of a product. Please comment specifically on whether the use of products containing chelating agents is likely to affect the level of antimicrobial activity of solid metal and metal alloy products. Also, please comment specifically on whether the cleaning step (thoroughly rinse with DI water) between exposure cycles is sufficient to remove residual chemical solutions (solutions A, B and C).

5. Residual/Continuous Activity: Residual/continuous activity over time is claimed to be one attribute inherent to copper and copper alloy products.
    a. Some technology developers would like to claim that solid copper and copper alloy products provide "residual/continuous activity." Please comment on whether the proposed protocol is capable of determining if copper and copper alloy products provide such activity, and if not, what changes to the proposed protocol (e.g., instituting repeated inoculations of the carrier) would provide data to evaluate such activity.

Appendix A
Overview of Changes to the Copper Protocol

1. **Test Microbes and Culture Preparation**: *Enterobacter aerogenes* as a Gram negative bacterium was not deemed essential to support the sanitizer claim and was removed from the protocol. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were retained. The preparation of test cultures was revised and is now consistent with the OECD Quantitative Method for Bacteria; Tryptic Soy Broth is used as the growth medium. (Refer to EPA Standard Operating Procedure MB-25: OECD Quantitative Method for Evaluating Bactericidal Activity of Microbicides Used on Hard, Non-Porous Surfaces)
2. **Quality Control**: A statement indicating that all aspects of testing must be conducted using Good laboratory Practice Standards was added to the protocol.
3. **Copper Product Attributes**: Observations of surface characteristics may be indicated as qualitative and/or quantitative assessments.
4. **Abrasion/Exposure Cycles**: The abrasion/exposure cycles were revised to include an 8 week period rather than a 12 week period. In addition, the initiation of the efficacy evaluation was revised to state with three days of the last abrasion/exposure cycle.
5. **Details for Supplies and Materials**: Sources have been provided for several reagents including the sodium hypochlorite, abrasion boat, spray bottle and the lint free cloth. The protocol has been revised to replace the abrasion pad on a daily basis, and 95-98% ethanol has been specified for use in the preparation of test carriers.
6. **Soil Load**: A three-part soil load has been specified as an addition to the test inoculum prior to carrier inoculation. The three-part soil load is consistent with the soil load requirement in the OECD Quantitative Method for Bacteria and serves to represent the soil burden found on environmental surfaces.
7. **Continuous Claim**: The protocol as written can be used to support a “continuous reduction” claim.
8. **Contact Time**: The revised protocol provides for a contact time of 1 hour, and for less than one hour upon consultation with EPA.
9. **Chelating Agent Activity**: The potential effects of chelating materials on copper/copper-alloy surfaces is under consideration.