Consumer Specialty Products Association

June 28, 2010

Office of pesticide Programs (OPP)
Regulatory Public Docket (7502P)
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460-0001

RE: Docket Control Number EPA-HQ-OPP-2009-0681 – Draft Test Guidelines

Dear Sir/Madam:

These comments are submitted on behalf of the Consumer Specialty Products Association (CSPA) regarding the four draft test guidelines for Product Performance of Public Health Use of Antimicrobial Agents – 810.2000, 810.2100, 810.2200 and 810.2300. We have made comments in the four drafts, which are attached with red-lined tracking. We also offer the following overall and specific comments on the four drafts.

1. **810.2000**

Overall Comments

- The 91 Guideline Series should be made available on the EPA website for users to access since portions of it are still relevant for supporting label claims. The Agency has not yet defined which sections of the 91 Guideline Series will not be included in the 810 Guideline series.

- Test organisms should always be cited throughout the document with an identifier (e.g., ATCC number) where one is defined.

- CSPA requests a description (e.g. title, description of future content) for all sections labeled as "Reserved" and that these sections not be left open without clarification.

- In appropriate places throughout the document, the term "should" was replaced with "must" as the term "should" may leave the document open for too much interpretation.

- This guideline describes in general the definitions and categories relevant to both public health and non-public antimicrobial agents. Since the 810.2000 Guideline is positioned as an overview document describing how the Agency views all antimicrobial agents, the title should be consistent and be changed to "General

Considerations for Uses of Antimicrobial Agents." Each individual guideline will then address the appropriate public or non-public health antimicrobial category.

- Since the Agency has not begun the process of updating the non-public health methods, this document should clearly point to Guideline Series 91B for the methods needed to support these products. CSPA requests that the Guideline Series 91B document be posted on the Agency website and that the Agency consider the inclusion of non-public health test guidelines in future revisions of the 810 series. The Agency is requesting the submission of non-public health data on a more frequent basis. CSPA requests the Agency update all non-public health guidelines.

- The 810.2000 Guideline is lacking a discussion on the emerging pathogen policy and CSPA requests this policy be added to this document.

- CSPA requests that the Agency define the terms "Volatile" and "Non-volatile" for inclusion in the 810.2000 Guideline. This information needs to be consistently migrated to each of the 810 Guideline series documents.

Specific Comments

(b)(4) Series Organization, Table 1.
The chart references Antimicrobials for use on Textiles (810.2400) and Antimicrobials for use in the Air (810.2500). CSPA has previously submitted comments on these Guidelines and are resubmitting the drafts to accompany these comments for Agency review.

(b)(5) Future guidelines
CSPA supports the Agency goal of updating these Guidelines periodically. These updates, however, must be fully vetted through a public notice and comment period.

As reflected in the Guideline, EPA may approve new methods through the internal protocol review process on a case by case basis (i.e., Internal Guidance for the Efficacy Protocol Review Process, http://www.epa.gov/oppad001/efficacyproto.htm). These methods must be published on the EPA website until such time as it can be added to these Guidelines.

Text surrounding the adoption of AOAC methods was added to the Guideline because CSPA's user community requests that adoption of an AOAC method by EPA should not occur until the method has achieved final status. The AOAC process is robust and provides not only for validation, but time to ensure that in practice the method does not exhibit any significant problems prior to final adoption; i.e. "Final Action". This final step, a two year process, is important in developing an understanding that a method will work consistently across a variety of labs. For this reason, CSPA encourages EPA not to adopt any AOAC method that is not in Final Action.

(c)(1)(iii)(B) Antimicrobial product with public health uses

The assertion that all disinfectants are considered to be human health-related, whether or not control of infectious microorganisms as specifically claimed is not correct. As noted in (c)(1)(iii)(C) and (c)(2)(i), product claims for non-public health (e.g., industrial water systems, material preservatives, veterinary and animal premises) use sites do not require submission of efficacy data; e.g they are non-public health products. Therefore, products can be registered that disinfect against animal organisms[1] only, odor-causing bacteria or other similar non-public health disinfectant claims. Therefore, the statement that all disinfectants are public health products is not correct.

(c)(1)(iii)(C) (Mold Remediation)
CSPA requests that the Agency insert a placeholder for mold remediation products. The Agency has been working on a public health mold remediation policy for some time and this information should be reflected in the Guideline series when completed. A place holder has been included in Table 1 for reference purposes.

(c)(2)(B) Bacteriostatic products
Since the Agency recognizes fungistatic products as a non-public health category, this section should be expanded to include fungistats (Section 93-15). The title of this section has been updated to reflect this change.

(c)(2)(D) Animal disease pathogens and zoonotic microorganisms
This section states "...the Agency is requesting the submission of efficacy data to support these claims because these pathogens have animal health significance or the potential to infect humans." The "or" needs to be changed to "and". As noted in (c)(2), the Agency does not require submission of non-public health data (i.e. animal health use patterns). Thus, the need to submit animal pathogen data is predicated on the potential to infect humans. In addition, CSPA has added a reference to a list of organisms that meet the criteria for which testing is required. This website has been previously given to CSPA for this purpose and is captured here as a reference.

(d) Definitions
CSPA strongly believes that all definitions provided must agree with those definitions provided in FIFRA; e.g. antimicrobial pesticide, disinfectant, etc. For those terms not defined in FIFRA, definitions consistent with those found in standard methods employed in testing antimicrobials should be used. Using legislated or standard recognized definitions avoids conflicting definitions that could lead to confusion. The attached revised 810.2000 Guideline provides references for the affected terminology.
In addition, CSPA has provided definitions for additional terms used in the Guidelines; e.g. slime, use/re-use, and limited disinfectant. The statement "For the purposes of these guidelines, slimicide claims are reserved for non-public health industrial label claims" is not appropriate. In a presentation made by Joan Harrigan-Farrelly at the 2008 CSPA Annual Meeting, CSPA was informed that the Agency had decided to not limit the use of the term "biofilm" to public-health related products or the use of the term "slime" to non-

[1] We recognize that those organisms known to have the capability of "jumping" from animals to humans are considered public health. However, a registrant could elect to have a product positioned that only claims organisms relevant to animals.

public health products. Instead, when using the term "biofilm" or "slime" in labeling, the use site will determine whether the Agency will require submission of efficacy data.

(e)(1) Test Substance
EPA has proposed language requiring that all efficacy studies be conducted at the lower certified limit (LCL). CSPA finds this language technically problematic. As written, we are concerned that registrant efficacy studies could be rejected if each active in the test substance is not at its LCL.

For products that contain multiple actives, actives that may have inverse relationships (e.g. peracetic acid/peroxide)[2], or the active is created *in-situ* producing test batches with all actives at the LCL would be difficult and quite possibly not achievable. Simple dilution is not the answer since 1) if there are multiple actives there is still difficulty in achieving LCL for each active and 2) dilution to achieve LCL could affect the ratio of the inert ingredients thus potentially affecting the efficacy profile of the product.
CSPA strongly urges the Agency to revert to the language used in Guideline 91-1 until CSPA has the opportunity to meet with the Agency to discuss their technical concerns with the proposed language.

(e) (ii) General testing considerations
This section has been updated to include the word "reporting" since this section contains language pertaining to both testing and reporting requirements.

f(3) Surrogate microorganisms
For the HBV, HCV and Norovirus studies, the fact that the data consistency controls found in the methods posted on the EPA web site are no longer required should be reflected in this guidance.

(g)(4) Exposure period
The Agency routinely accepts shorter time periods than is listed in methods. This section is worded in a way that makes it seem like registrants must seek Agency concurrence before conducting studies with shorter time periods. This is burdensome for both the Industry and the Agency when current policy allows time reductions on a routine basis.

(g)(5) Use/Reuse of products
The proposed Guideline does not include any reference to use/reuse studies. CSPA has proposed re-instating the language for products that may carry this claim. While use/reuse is most commonly associated with reprocessing of medical equipment, an FDA regulated claim, there may be instances where use/reuse is indicated for EPA applications. Thus, registrants want to maintain the right to have this use on EPA regulated products.

(g)(6) Confirmatory Testing

[2] While peracetic acid and peroxide reach equilibrium, the level of oxidant is distributed between the two species. Thus, an increase in one species results in a decrease in the other species. When the actives are analyzed, it reflects the position of the equilibrium at that time.

Language surrounding Confirmatory testing, Duplicated product formulations and Minor formulation change in a registered product were migrated to the 810.2000 Guideline from 810.2200 and 810.2300.

The Agency also indicates that the Germicidal Spray Test must be used with liquid products containing volatile active ingredients. No guidance is provided on what is meant by volatile active ingredients and thus should be deleted.

(h)(1)(3) Neutralization
Considering that Neutralization is a key part of any antimicrobial study, CSPA requests that a more detailed description of neutralization techniques and its importance be highlighted in this document. CSPA has proposed language surrounding neutralization and some examples that could be used for reference.

(h)(6) Test failure
It is important that registrants have clear guidance on what to do in the case of a product failure. A low level failure in a particular batch or a system (human) error can occur during the course of running a study. This does not necessarily indicate the overall product performance of a particular formulation. In addition, the AOAC Use-dilution Test Method allows for repeating an assay to confirm for the presence of false positives. CSPA has included language as previously discussed an agreed with the Agency as acceptable common practice to clarify the process.

(i)Data collection and reporting
To assist in the proper review and evaluation of product performance, CSPA recommends including a section describing what information should be included in a final report. Proposed language is included with our Guideline revision. This information was also removed from other 810 Guideline documents and consolidated to 810.2000 for convenience.

2. 810.2100

Overall Comments

- The 91 Guideline Series should be made available on the EPA website for users to access since portions of it are still relevant for supporting label claims. The Agency has not yet defined which sections of the 91 Guideline Series will not be included in the 810 Guideline series.

- Test organisms should always be cited throughout the document with an identifier (e.g., ATCC number) where one is defined.

- CSPA requests a description (e.g. title, description of future content) for all sections labeled as "Reserved" and that these sections not be left open without clarification.

- ❖ CSPA requests that the Agency define the terms "Volatile" and "Non-volatile" for inclusion in the 810.2000 Guideline. This information needs to be consistently migrated to each of the 810 Guideline series documents.

- Currently the 810.2100 Guideline document does not contain a table which outlines the testing requirements for the public health efficacy claims. This table exists in the other 810 Guideline series documents and needs to be included in 810.2100 for consistency.

- In appropriate places throughout the document, the term "should" was replaced with "must" as the term "should" may leave the document open for too much interpretation.

- Throughout the document test procedure sections, references were made to a target carrier count range of $1 \times 10^5 - 1 \times 10^6$ colony forming units per carrier. CSPA removed these carrier count ranges considering that this range has not been collaborated, validated or been subject to an SAP. It is our understanding that not all organisms covered by this Guideline can achieve this range as it is very dependant on carrier type and organism. Though, we applaud the Agency for inclusion of a range and CSPA is in support of this for the future, we request that the ranges included be fully validated before publishing a range that is not achievable.

In addition, if the Agency re-inserts this language, CSPA requests that throughout the document the Agency remove the word "approximately" when referencing carrier counts. For example: (ii) Test procedure for sporicidal decontaminants – qualitative testing. "The inoculum employed must provide a target count of 1×10^5 – approximately 1×10^6." It is unclear as to how the term "approximately" is applied in this instance.

- The 810.2100 Guideline is missing information on the use of porous carriers as the more stringent test standard. In 810.2000, (g)(1)Type of Surface, a hard, porous carrier may be substituted for a non-porous carrier. The statement is as follows:
"Since the use of a hard, porous surface would simulate the more stringent test condition, demonstrated efficacy on hard, porous surfaces would suffice to support an analogous claim for efficacy of the product on hard, non-porous surfaces as well."

Specific Comments

(a) (d)(1)Test Procedure
Official Methods of Analysis of AOAC international, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method II is limited to use for B. subtilis spores dried on porcelain penicylinders. Method II has not been validated by AOAC for B. subtilis spores dried on suture loops and C. sporogenes dried on porcelain penicylinders and suture loops. Current AOAC reports on this effort have indicated that there are difficulties in achieving this validation. Based upon the current status of the validation attempts, CSPA recommends that EPA continue to allow the use of Method I.

(2) Evaluation of sterilant success

An explanation of the term "failures" has been included to clarify the language. This reads "... growth of test organism after carrier treatment"

(d)(2)(e) Validation testing for all products with sterilant claims. CSPA requests the Agency adopt the same policy used for surrogate organism testing and allow for the independent validation testing to be done in either a separate facility or in the same facility as the initial testing but with separate staff. This has been acceptable in the past and needs to be adopted across all testing categories for all organisms. This will allow for the testing to be done consistently and does not detract from the overall efficacy evaluation of the product.

(g) Additional spore formers, *Clostridium difficile* (*C. difficile*) claims
This section addresses interim guidance for products. CSPA does not believe this guidance qualifies as "interim" and has removed this language from the Guideline.

(g)(1)(i) Water-soluble powders and liquid products, qualitative testing
Official Methods of Analysis of AOAC international, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method II is limited to use for B. subtilis spores dried on porcelain penicylinders. Method II has not been validated by AOAC for B. subtilis spores dried on suture loops and C. sporogenes dried on porcelain penicylinders and suture loops. Current AOAC reports on this effort have indicated that there are difficulties in achieving this validation. Based upon the current status of the validation attempts, CSPA recommends that EPA continue to allow the use of Method I.

Currently, AOAC Method II specifies a spore purity limit; however, it is unclear as to how this is accomplished without specific guidance. CSPA requests the Agency clarify how to achieve this spore purity limit and whether this applies to Method I as well.

(h)(1) Water-soluble powders, liquid products, gases and vapors – (i) Test procedure for sterilant / sporicide plus B. anthracis claim.
CSPA requests the Agency provide a list of surrogate organisms appropriate in place of *B. anthracis*. CSPA has recommended the use of *B. subtilis* ATCC 19659 as a suitable surrogate.

(h)(1)(iii) Test procedure for sporicidal decontaminants – quantitative testing.
CSPA requests the Agency provide guidance on what surrogate organism would be appropriate in place of *B. anthracis*. CSPA has recommended the use of *B. subtilis* ATCC 19659 as a suitable surrogate.

(2)(ii)(D) Additional Considerations
CSPA requests the Agency better define the statement "appropriate positive and negative controls should employed."

(h)(iii) Test procedure for sporicidal decontaminants--quantitative testing.
CSPA requests a rationale for how the target count of 1×10^7 colony forming units per carrier was determined and whether it has been collaborated and validated.

(j) Data Collection and Reporting
This section was moved to 810.2000 for consistency.

(1) General
This section was moved to 810.2000 for consistency

(2) Data for recommended methods.
This section was moved to 810.2000 for consistency.

3. 810.2200

Overall Comments

- The 91 Guideline Series should be made available on the EPA website for users to access since portions of it are still relevant for supporting label claims. The Agency has not yet defined which sections of the 91 Guideline Series will not be included in the 810 Guideline series.

- Test organisms should always be cited throughout the document with an identifier (e.g., ATCC number) where one is defined.

  In section (g) (1) (i), the name for Salmonella typhi (ATCC 6539) has been changed to Salmonella enterica (ATCC 6539). In section (j)(ii)(B) the ATCC strain numbers needs to be identified.(i) Sanitizers for Toilet and Urinal Bowl Water , section (iii) an upper limit or range needs to be identified for the organism (inoculum) control.

- CSPA requests a description (e.g. title, description of future content) for all sections labeled as "Reserved" and that these sections not be left open without clarification.

- In appropriate places throughout the document, the term "should" was replaced with "must" as the term "should" may leave the document open for too much interpretation.

- The minimum mean log density for *S. aureus* and *P. aeruginosa* has been defined in the 810.2200 document; however, an upper limit has not yet been assigned. CSPA welcomes the opportunity to provide support to the EPA for establishment of a standard carrier count range for EPA required methods that do not currently contain instructions for culture standardization. Discussions are ongoing as to the proper carrier count ranges that are appropriate for each method. Laboratories may find that proposed carrier counts are easily achievable using the AOAC culturing instructions or that it may be necessary for the culture to be standardized to achieve the proposed range. The steps required to achieve the range must be detailed in the study final report.

-Documents, references and methods cited in 810.2200 should be made publically available. For example: Sanitizer for Urinal and Toilet Bowl Water and in-tank Sanitizers, EPA Sanitizer Test, 1976 and any unpublished DIS/TSS documents.

References to specific test methods where the current edition was provided were modified to remove the edition number since it needs to be communicated to the user community that standard practice needs to be to use the most recently revision.

Sections of the document are divided by whether a test product is considered volatile or non-volatile. These designations need to be defined further in order to completely assess whether the test criteria are appropriate for that particular chemistry. These definitions need to be included in 810.2000 for consistency. If this cannot be defined, then the language needs to be removed from the document.

CSPA believes that the use instructions for the disinfectant testing and the success criteria need to be better defined. For example: towelette carrier size and success criteria evaluation. This has been defined in the 810.2200 document as red line additions.

CSPA welcomes the opportunity to provide support to the EPA for establishment of a standard carrier count range for EPA required methods that do not currently contain instructions for culture standardization. Discussions are ongoing as to the proper carrier count ranges that are appropriate for each method. Laboratories may find that proposed carrier counts are easily achievable using the AOAC culturing instructions or that it may be necessary for the culture to be standardized to achieve the proposed range. The steps required to achieve the range must be detailed in the study final report.

In addition, the information provided allowing for the use of the Hard Surface Carrier Test needs to be further defined in the success criteria to include the carrier count requirements for each test organism.

The information provided on success criteria is not consistent across each of the sections. This language needs to be better defined and made consistent to avoid confusion.

Specific Comments

(3) Confirmatory Testing
This section was moved to 810.2000 for clarity and consolidation of language.

(C)(4) Table 1
The table was revised to add clarification on test method references, number of batches/carriers required, test organism requirements and acceptance criteria to be consistent with current guidelines and practices. The table requires additional revisions to make sure that all possible levels of efficacy are taken into consideration. This includes confirmatory testing requirements for Tuberculocides and Fungicides and to add in information on suspension tests where they are applicable.

(2) Confirmatory testing for limited spectrum products (iv) Evaluation of confirmatory limited disinfectant success.

The mean log density was supplied for *S. aureus* only. The information corresponding to *S. enterica* was added as clarification.

The mean log density for *S. aureus* is listed as at least 6.0 (corresponding to a geometric mean density of 1.0×10^6); a mean log density <6.0 invalidates the test. This information as found in AOAC is not finalized. CSPA requests clarification as to whether this is an EPA requirement even though the corresponding test method is not a Final Action.

From 810.2000 Guideline comments:
Text surrounding the adoption of AOAC methods was added to the Guideline because CSPA's user community requests that adoption of an AOAC method by EPA should not occur until the method has achieved final status. The AOAC process is robust and provides not only for validation, but time to ensure that in practice the method does not exhibit any significant problems prior to final adoption; i.e. "Final Action". This final step, a two year process, is important in developing an understanding that a method will work consistently across a variety of labs. For this reason, CSPA encourages EPA not to adopt any AOAC method that is not in Final Action.

(iii) Single-use towelettes test procedure.
CSPA requests that the Agency define the success criteria to accommodate the larger size carrier for towelette testing. For example: how many total carriers per batch, what level of efficacy is required, etc. EPA needs to define if the larger carrier size is considered a new method for which validation has not yet been performed.

(8) Disinfectants for Internal Toilet and Urinal Bowl Surfaces Above and Below the Water Line.
The Agency needs to define the total volume to be used for calculation of the appropriate use dilution used for testing of toilet bowl products. The current guidance requires the use of ~96 fl oz as representative of a typical toilet. With the inclusion of low volume toilets in homes the total volume needs to be adjusted to account for a change in consumer use.

(e) Disinfectants with fungicidal claims. (1) Water soluble powders and non-volatile liquid products; (i) Test Procedures
The inoculum employed for Fungicidal activity currently specifies a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. This level has not been validated, collaborated or discussed through an SAP process. How was this level derived?

(3) Single-Use Towelettes; (i) Test Procedure
The inoculum employed for Fungicidal activity currently specifies a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. This level has not been validated, collaborated or discussed through an SAP process. How was this level derived?

(iv) Evaluation of disinfectant success for additional microorganisms.
Currently, the minimum carrier count and therefore success criteria for efficacy against additional organisms is not defined. CSPA requests the Agency provide guidance based on discussions with industry.

(4) Evaluation of virucidal success
This section was modified to account for changes to current practices. For example:

- Changes in testing for surrogate organisms including removal of control testing requirements.
- Changes in standard industry practice for calculation of the reduction in virus titer by inclusion of alternative statistical methods.
- Providing additional examples of results and methods of calculation.

(g) Multiple-Use Towelette
The Multiple-Use Towelette language was added from DIS/TSS.

(h) Data Collection and Reporting
This section was moved to 810.2000 for consistency.

(1) General
This section was moved to 810.2000 for consistency

(2) Data for recommended methods.
This section was moved to 810.2000 for consistency.

4. **810.2300**

Overall Comments

- The 91 Guideline Series should be made available on the EPA website for users to access since portions of it are still relevant for supporting label claims. The Agency has not yet defined which sections of the 91 Guideline Series will not be included in the 810 Guideline series.

- Test organisms should always be cited throughout the document with an identifier (e.g., ATCC number) where one is defined.

  In section (g) (1) (i), the name for Salmonella typhi (ATCC 6539) has been changed to Salmonella enterica (ATCC 6539). In section (j)(ii)(B) the ATCC strain numbers needs to be identified.(i) Sanitizers for Toilet and Urinal Bowl Water , section (iii) an upper limit or range needs to be identified for the organism (inoculum) control.

- CSPA requests a description (e.g. title, description of future content) for all sections labeled as "Reserved" and that these sections not be left open without clarification.

- In appropriate places throughout the document, the term "should" was replaced with "must" as the term "should" may leave the document open for too much interpretation.

- ❖ CSPA welcomes the opportunity to provide support to the EPA for establishment of a standard carrier count range for EPA required methods that do not currently contain instructions for culture standardization. Discussions are ongoing as to the proper carrier count ranges that are appropriate for each method. Laboratories may find that proposed carrier counts are easily achievable using the AOAC culturing instructions or that it may be necessary for the culture to be standardized to achieve the proposed range. The steps required to achieve the range must be detailed in the study final report.

-Documents, references and methods cited in 810.2300 should be publically available. For example: Sanitizer for Urinal and Toilet Bowl Water and in-tank Sanitizers, EPA Sanitizer Test, 1976 and any unpublished DIS/TSS documents.

Specific Comments

(C)(3) Efficacy Claims, Table 1
The table was revised to add clarification on test method references, number of batches/carriers required, test organism requirements and acceptance criteria to be consistent with current guidelines and practices. The table requires additional revisions to make sure that all possible levels of efficacy are taken into consideration.

DIS/TSS 4 requires S. typhi for Food Contact Testing of Halide Products. Table 1 of the 810.2300 states test organisms for the Food Contact Testing of Halide Products as Salmonella typhi OR S. aureus. Please clarify if the intent is to provide two options of test organisms.

(f)(1) Test Procedures
The Agency needs to define the total volume to be used for calculation of the appropriate use dilution used for testing of toilet bowl products. The current guidance requires the use of -96 fl oz as representative of a typical toilet. With the inclusion of low volume toilets in homes the total volume needs to be adjusted to account for a change in consumer use.

(2)(1)(iv) Microbial counts of the treated bowl water
The Agency needs to define the three exposure intervals as mentioned in the text including giving clear guidance on how these were derived and what they equate to (e.g., duration, tablet lifespan, etc.).

(k)(1) Test Procedure
The Residual self-sanitizing products information needs to be expanded to describe the carrier type and number of carriers tested as per the method. This section lacks clarity as to how the method is to be run.

(m) Data Collection and Reporting
This section was moved to 810.2000 for consistency.

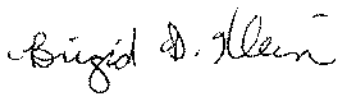
(1) General
This section was moved to 810.2000 for consistency

(2) Data for recommended methods.
This section was moved to 810.2000 for consistency.

Conclusion

CSPA appreciates the opporutnity to comment on the draft test guidelines for Product Performance of Public Health Use of Antimicrobial Agents. If you have any questions on these comments please contact me at 202-833-7309.

Sincerely,



Brigid D. Klein
Vice President & General Counsel

CSPA COMMENTS on 810.2000
6 28 2010

OPPTS 810.2000: General considerations for ~~use~~testing ~~of public health~~ antimicrobial agents.

(a) Scope.

(1) Applicability. This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21U.S.C. 346a).

(2) Background. The source material used in developing this OPPTS test guideline is OPP guideline 91-1: General Requirements for Antimicrobial Agents (Pesticide Assessment Guidelines, Subdivision G, Product Performance, EPA report 540/9-82-026, October 1982).

(b) Overview—Product performance.

(1) General concepts. Any evaluation of product performance is conducted in light of expressed and implied labeling claims or recommendations concerning pests, sites, methods of application, application equipment, dosage rates, timing and number of applications, use situations, nature and level of pest control, duration of pest control, compatibility with other chemicals, benefits and/or adverse effects of product use, compatibility of common practices associated with the sites, active ingredient status of chemicals in the formulation, and equipment.

(i) Laboratory and/or simulated-use testing is conducted to determine the effectiveness of a substance, or mixture of substances, to control or kill specific pest organisms, and in some cases to determine whether the substance has sufficient pesticide potential to warrant larger scale testing (e.g., swimming pool disinfectants).

(ii) In some cases, effectiveness ~~and usefulness~~ of the proposed product is further proven through advanced large-scale laboratory tests, field tests, in-use tests, or simulated-use tests by procedures which closely approximate actual use and which employ typically used application equipment (e.g. fumigant sterilants).

(2) [Reserved]

(3) Waiver policy. As outlined in 40 CFR Part 158, the Agency has waived all requirements to submit efficacy data unless the pesticide product 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. However, pursuant to FIFRA, each registrant must ensure through testing that his products are efficacious when used in accordance with label directions and commonly accepted pest control practices. The registrant must develop and maintain the relevant data upon which the determination of efficacy is based. The agency reserves the right to

require, on a case-by-case basis (e.g., zoonotic microorganisms) submission of efficacy data for any pesticide product, registered or proposed for registration.

(4) Series organization. Table 1 outlines the organization of the OPPTS Test Guideline Series 810.2000.

Table 1. Organization of the OPPTS Test Guideline Series 810.2000.

Pesticide Type	Guideline Number	Previous Subdivision –G Guideline Number(s)
Antimicrobials for use as Sterilants	810.2100	91-2(a)
Antimicrobials for use as Disinfectants, Fungicides, Virucides, & Tuberculocides	810.2200	91-2(b)(c)(d)(e)(f)(g)(i) 91-7(a)(1) 91-3
Antimicrobials for use as Sanitizers – Food & Non-Food Contact Surfaces, Residual	810.2300	91-2(j)(k)(l)(m) 91-3
Antimicrobials for use on Textiles	810.2400	91-4(a)(1)(2)(3)(4) 91-4(b)(c)(d)
Antimicrobials for use in the Air	810.2500	91-5
Antimicrobials for use in Water (Swimming pool, Drinking water)	810.2600	91-8
Mold Remediation	810.xxxx	Not Applicable

(5) Future guidelines. The Agency recognizes the fact that novel technologies associated with antimicrobial products may evolve over time and would potentially involve test methods that are not referenced in this current guideline. In addition, the Agency is considering adopting the use of quantitative test methods ~~as a possible replacement for current qualitative methods [e.g., Association of Official Analytical Chemists (AOAC) Use-Dilution Methods] in the future~~ The Agency regards these guidelines as living documents and intends to ~~periodically~~ update these ~~guidelines periodically.~~documents through public notice and comment periods as dictated by changes in science and policy. However, the use of new methods may be approved, on a case-by-case basis, prior to guideline updates and will be published on the EPA Antimicrobial Policy and Guidance Documents website until such time that they can be added to the guidelines. When EPA elects to adopt an AOAC method as part of a guideline, the method must have Final Action status.

(c) Public health and nonpublic health uses of antimicrobial products

(1) Antimicrobial products with public health uses. (i) Health-related considerations. Microbial pests can be categorized into two basic types: Those that present potential public health hazards because of their infectious nature to humans and those that cause economic or aesthetic problems such as spoilage, fouling, or production of offensive odors in the substrate in which they grow. The OPPTS Test Guideline Series 810.~~2000~~ 2100 - 2600 address

antimicrobial pesticide products with public health uses for which efficacy test data are required to be submitted to support registration. These include all antimicrobial products intended to control microorganisms infectious to man in any area of the inanimate environment where these microorganisms may present a hazard to human health. The label claims for an antimicrobial product determine whether it is considered to be related to human health.

(ii) Products bearing claims to control organisms that may pose a threat to human health, either directly or through transmission of disease-causing organisms on environmental surfaces or the environment, are considered public health related antimicrobials, and require specific efficacy data to support labeling claims and patterns of use. Unqualified and non-specific claims for products as sterilants, disinfectants, or sanitizers are considered to include or imply effectiveness against microorganisms infectious to man. Antimicrobial products recommended for use in hospital or medical environments, including but not limited to; sickrooms in public or private dwellings, are similarly considered as human health-related. Such claims or recommendations need to be expressly qualified or deleted in order to remove implications of human health significance.

(iii) Products of human health significance. The types of products in paragraphs (c)(1)(iii)(A) and (c)(1)(iii)(B) in this guideline are considered to be of human health significance.

(A) Products bearing labeling claims to control specific microorganisms that are infectious for man on/in environmental surfaces or the environment, such as *Staphylococcus aureus, Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa*, are considered to be directly related to human health.

(B) All public health products including sterilants, disinfectants, swimming pool water disinfectants/sanitizers, human drinking water disinfectants and purifiers, and food-contact surface sanitizers are considered to be human health-related, whether control of infectious microorganisms is specifically claimed. Non-public health products are those specified in (c)(2).

(C) Mold Remediation – Reserved

(2) Antimicrobial products with nonpublic health uses. Registrants who propose non-health related claims for their product (e.g., control of odor-causing bacteria) should be aware that generally the Agency does not require submission of efficacy data to support such claims. However, the registrant is still responsible for ensuring that these products perform as intended by developing efficacy data which must ~~should~~ be kept on file. The Agency still has the responsibility of making sure that the use directions proposed for non-public health related claims are appropriate and adequate. Therefore, the Agency retains the option of requiring the submission of efficacy data for non-public health related claims on a case-by-case basis (e.g., fabric and hard surface mildewstat and mold remediation studies for which guidance is forthcoming.) The types of products in paragraphs (c)(2)(A) through (c)(2)(D) in this guideline are considered to be non-public health related products.

(A) Slime, odor control and other non-public health agents. Slime and odor control

agents, preservatives, algicides, and other products expressly claiming control of microorganisms of economic or aesthetic significance are not considered to be human health-related, but nevertheless must bear accurate labeling claims and adequate dosage recommendations, and complete directions for use.

(B) Bacteriostatic/Fungistatic products. Since elimination or significant reduction in the number of microorganisms (sterilization, disinfection, sanitization) must ~~should~~ be demonstrated before a product is considered acceptable for use against microorganisms infectious for humans, or for use in medical or sickroom environments, products bearing labeling claims for effectiveness at the bacteriostatic/fungistatic (inhibition of growth) level are not appropriate for such uses. Bacteriostatic/fungistatic claims are generally only acceptable for products expressly recommended for control of microorganisms of aesthetic significance (e.g., spoilage bacteria, odor-causing bacteria, mold and mildew).

(C) Treated articles. The Agency has clarified its policy on applicability of the treated articles exemption to antimicrobial pesticides and provided guidance on appropriate language or label claims in Pesticide Registration Notice 2000-1 (see reference (i)(1) of this guideline). The exemption (40 CFR 152.25 (a)) covers qualifying articles and substances bearing claims to merely protect the article or substance itself, if the pesticide is registered for such use. This exemption does not include articles or substances bearing implied or explicit public health claims against human pathogens. Applicants who intend to market products with claims (such as public health claims) that go beyond the scope of the treated articles exemption should contact the Antimicrobials Division prior to conducting testing to support this use.

(D) Animal disease pathogens and zoonotic microorganisms. For products labeled for public health and/or non-public health uses, submission of studies to EPA on certain animal disease pathogens and zoonotic microorganisms may be required prior to approval of the label claim. For example, although label claims against foot and mouth disease virus, Newcastle disease virus, and avian influenza A virus are not considered to be human health related, the Agency is requesting the submission of efficacy data to support these claims because these pathogens have animal health significance and ~~or~~ the potential to infect humans. Applicants should consult the Agency for a current listing of organisms which meet these criteria. See, http://www.oie.int/eng/maladies/en_classification.htm.

(d) Definitions. Because of the variety of microorganisms to be controlled and the different claims and many use patterns of antimicrobial products, uniform product terminology and a common understanding of a few key words are important to a program for evaluating product performance. Even though the OPPTS Test Guideline Series 810.~~2000 guidelines~~ cover only public health uses, terms covering non-public health use patterns and/or organisms are included in order to support consistency and clarity in the regulations of antimicrobial pesticides. The terms in the OPPTS Test Guideline Series 810.~~2000~~ are generally used with the meanings set forth in this paragraph.

Algicide means any substance, or mixture of substances, which is intended to kills or effectively reduces the number of living algae in water.

Algistat means any substance, or mixture of substances, that is intended to inhibits the growth of algae in water.

Antibacterial means any substance, or mixture of substances, that destroys or eliminates bacteria in the inanimate environment.

Antibiotic resistant means the organism is not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and / or fall in the range where specific microbial resistance mechanisms are likely (e.g., beta-lactamases), and clinical efficacy has not been reliable in treatment studies. ~~*Antibiotic resistant* means the~~ (Ref. 23) ~~ability of a bacterial cell to resist the effects of antibiotics.~~

Antifoulant means any substance, or mixture of substances that is used to prevent the biological fouling of underwater structures or objects.

Antimicrobial Pesticide means a pesticide (substance or mixture of substances) that is intended to disinfect, sanitize, reduce or mitigate growth or development of microbiological organisms or protect inanimate objects, industrial processes or systems, surfaces, water or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae, or slime (FIFRA § 2 (mm)).

Antiseptic means a drug product applied topically to the skin or mucous membranes to help prevent infection or to help prevent cross contamination. Antiseptic products are applied on or in the living body of man or other animals. Antiseptic products are not identified as pesticides and are regulated by the Food and Drug Administration.

Aseptic means free of microbial contamination consistent with FDA 21 CFR 178 for commercial sterilants for aseptic food packaging or an effective Food Contact Notification (FCN).

Bacteriostat means a substance, or mixture of substances that inhibits the growth of bacteria in the inanimate environment.

Biocide/ Microbiocide mean any substance, or mixture of substances, that kills a number of living microorganisms (e.g., virucide-virus, mycobactericide-mycobacteria, algicide-algae; bactericide-bacteria; fungicide-fungi; slimicide-slime-forming microorganisms). Note: The terms *bactericide* and *fungicide*, as used in conjuction with the term *microbiocide*, are only related to industrial uses.

Biofilm means ~~community of bacteria or other microorganisms encased in an extracellular polysaccharide substance that attach to a variety of substrates --~~ a dynamic, self-organized accumulation of microorganisms and environmental by-products immobilized on a substrate and embedded in an organic polymer mix (ASTM E35.15 Draft). This organic polymer mix is also known by the term "glycocalyx."

Confirmatory data is a reduced set of data which may be used to support an application or amendment for registration of a product, or a minor formulation change of a registered product.

Deodorizers means a substance, or mixture of substances that are of two basic types: (1) Those that prevent or delay the formation of bacterial odors by killing microorganisms which produce them, and (2) those that mask, chemically destroy, or neutralize odors. Products that claim deodorization by antimicrobial means are subject to registration as pesticides under FIFRA.

Disinfectant means a substance, or mixture of substances ~~that~~ intended to destroys or ~~eliminates a specific species of infectious or public health microorganism, but not necessarily bacterial spores, in the inanimate environment.~~irreversibly inactivate bacteria, fungi, or viruses on surfaces or inanimate objects (FIFRA § 4(i)(4)(c)(iii)).

- Limited Spectrum Products: the substance or mixture of substances is effective against Gram negative or Gram positive bacteria.
- ~~General or~~ Broad-Spectrum Efficacy Products: the substance or mixture of substances is effective against Gram negative and Gram positive bacteria.
- ~~Hospital or~~ Healthcare Disinfectants: the substance or mixture of substances is effective against Gram negative and Gram positive bacteria additionally including efficacy for *Pseudomonas aeruginosa* (ATCC 15442).
- ~~The~~ Gram negative and Gram positive bacteria required are as referenced in the OPPTS 810.2200 series.

Fungicide means a substance, or mixture of substances that destroys fungi (including yeasts) and/or fungal spores pathogenic to man or other animals in the inanimate environment.

Fungistat means a substance, or mixture of substances that inhibit the growth of fungi in the inanimate environment.

Microbiological water purifier means any unit, water treatment product or system that removes, kills, or inactivates microorganisms from the water, including bacteria, viruses and protozoan cysts so as to render the treated water safe for drinking.

Microbiostat means a substance, or mixture of substances, that inhibit the growth of microorganisms (e.g., bacteriostat, fungistat, algistat).

Mycobactericide means a substance, or mixture of substances, that destroys or irreversibly inactivates mycobacteria in the inanimate environment.

One-Step Disinfectant means a substance, or mixture of substances that has been tested and found to be effective in the presence of a light to moderate ~~bioburden~~ soil, and therefore, may be used without a pre-cleaning step in the use directions.

Preservative means a substance, or mixture of substances that inhibits the growth of microorganisms capable of causing biological deterioration of a material(s).

Product performance refers to all pesticidal aspects of a product's effectiveness and usefulness.

Use / Re-Use: The Recommended Methods are designed to demonstrate efficacy of a freshly prepared antimicrobial solution intended for a single application. When the same use solution is intended for repeated applications, testing must be conducted in accordance with a test protocol specially designed to demonstrate retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges (such as supplemental recommendations indicated above) and stress conditions (such as an inadvertent or incidental dilution inherent in the use pattern) over the period of time or number of times specified in the directions for use. (Ref. 18)

Sanitizer means a substance, or mixture of substances ~~that reduces the bacterial population in the inanimate environment by significant numbers, but does not destroy or eliminate all bacteria or other microorganisms~~ intended to reduce the number of microorganisms on inanimate surfaces, in water or air (FIFRA § 4(i)(4)(c)(i).

Slime means a dynamic, self-organized accumulation of microorganisms and environmental by-products immobilized on a substrate and embedded in an organic polymer mix (ASTM E35.15 Terminology Standard Draft). This organic polymer mix is also known by the term "glycocalyx."

Slimicide means a substance, or mixture of substances that reduces the number of slime-forming microorganisms ~~in industrial water systems (e.g., paper mills). (Ref. 21)For the purposes of these guidelines, slimicide claims are reserved for non-public health industrial label claims.)~~

Sterilant means a substance, or mixture of substances that destroys or eliminates all forms of microbial life in the inanimate environment, including all forms of vegetative bacteria, bacterial spores, fungi, fungal spores, and viruses.

Sporicide means a substance, or mixture of substances, that irreversibly inactivates bacterial spores in the inanimate environment.

Tuberculocide means a substance, or mixture of substances that destroys or irreversibly inactivates tubercle bacilli in the inanimate environment.

Two-Step Sanitizer or Two-Step Disinfectant means a substance or mixture of substances that has not been registered for effectiveness against microorganisms in the presence of a ~~bioburden~~ light to moderate soil. The sanitizer or disinfectant use directions should state the need to pre-clean surfaces prior to sanitizing or disinfecting.

Virucide means a substance, or mixture of substances that destroys or irreversibly

inactivates viruses in the inanimate environment.

Zoonotic microorganism means an infectious agent that can be transmitted between animals and humans.

(e) General testing / reporting considerations

(1) Test substance.

(i) Unless otherwise specified, antimicrobial pesticides must be tested on the formulation ~~with the lowest certified limit(s) of the active ingredient(s) and,~~ to be offered for sale and in some cases, (e.g., pressurized sprays, ~~towelettes~~) with the product in the same packaging as intended to be marketed.

(ii) The following information must be provided: ~~Identification should be made of the test substance and quantitative description of its chemical composition should be reported~~.

(A) Identification ~~should be made~~ of the test substance and quantitative description of the ~~it chemical~~ active ingredient(s) composition ~~must be reported~~.

(B) Manufacturer and production batch numbers of the test substance ~~should be reported~~. If a product is diluted, the report must specify the quantities and identification of each diluent.

(C) The manufacturer must ~~should~~ also submit effectiveness data to show that they can consistently reproduce the formulation (batch replication), as well as to show that the product will retain its effectiveness for a minimal period of storage under average conditions to which it is likely to be exposed. ~~(shelf life stability)(Ref.2).~~ To ensure a product can be consistently reproduced, the registrant is required to submit replicated batches. Refer to the 810 OPPTS Guidelines for batch replication requirements.

(D) For many pesticide products, data on similar formulations may be used to supplement data on a specific formulation. (Refer to: Subdivision G, Nov. 82, pg. 44).

(2) General considerations

(i) Good Laboratory Practice Standards. Antimicrobial products targeting public health pathogens and zoonotic microorganisms should be tested in accordance with the Good Laboratory Practice Standards outlined in 40 CFR Part 160 and following the proposed directions for use.

(ii) Use pattern. Depending upon the type of antimicrobial agent, target microorganisms, and the site to be treated, all tests should address those factors that would normally be expected to be encountered in the use pattern intended for the product, such as the method of application, the nature of the surface, item or substrate to be treated, the presence or absence of soil or other interfering conditions, temperature, exposure period, and the number of times or duration of time

that the use solution can be used or reused. Representative surfaces for testing are identified in each 810 Guidelines.

(iii) Additional factors. The actual test procedure to be employed will vary according to the characteristics of the product, the target pests and the pattern of use intended. A specification of methods in these guidelines for all conceivable public health uses is not feasible, and the applicant ~~should be~~ is responsible for the validity of the test method selected to substantiate a product's efficacy. The applicants should ensure themselves that the selected method is current and applicable to the product and uses proposed for registration.

(iv) New methods. If applicants believe they have alternative protocols for demonstrating the efficacy of a product, such protocols should be submitted to the Agency for review. In addition to modifying the standard methods, registrants may, in consultation with the Agency, develop and submit protocols for claims where no standard test methods have been developed.

(3) Use of Antibiotic Resistant Test Organisms. Organisms to be labeled as antibiotic resistant must ~~should~~ be accompanied by scientific data that substantiates the antibiotic resistance. The Antibiotic Resistance confirmation must ~~should~~ be conducted using the organism(s) listed on the label, and, if possible, should be performed at the same time as the efficacy testing. The confirmation may also be conducted within the usual transfer cycle or other appropriate transfer depending upon the organism's growth requirements. The information in paragraphs (e)(3)(i) through (e)(3)(iv) in this guideline must ~~should~~ be submitted from the Antibiotic Resistance Confirmation testing or included in the final report.

(i) Test organisms must ~~should~~ be characterized according to paragraphs (e)(3)(i)(A) through (e)(3)(i)(D) of this guideline:

(A) the source and identity (e.g. ATCC, private source, other).

(B) the method of preparation prior to testing (e.g. transfer history).

(C) the method used to confirm the identity (e.g. biochemical test, Gram stain, morphology).

(D) the method of preservation/storage (e.g. refrigerated agar slants, cryogenic beads, other).

(ii) Results of the testing including the numerical values of all antibiotics tested. An example of values would be Minimum Inhibitory Concentration (MIC) s for automated test, zone sizes for manual tests, and comparison to a standard National Clinical Laboratory Standards (NCCLS) Interpretation of such tests.

(iii) The scientific method used to obtain the results (Kirby-Bauer, disc agar diffusion, or gradient agar diffusion; automated MIC procedures or equivalent). If automated procedures are

used, the manufacturer of such automation should be specified.

(iv) Quality control procedures used to verify results.

(f) Special considerations

(1) Hard Surface Carrier Test vs. Use-Dilution Methods. The AOAC International Hard Surface Carrier Test Method (Ref. 3) has only been validated for use with distilled water. For other conditions (hard water, organic soil, and/or distilled water), the AOAC International Use-Dilution Method (Ref. 4) or the AOAC Germicidal Spray Products test are ~~is~~ the recommended methods.

(2) Elimination of Phenol Resistance Testing. As described in Pesticide Registration Notice 2001-4 (Ref. 5) the Agency is no longer recommending the use of the phenol resistance assay when conducting carrier-based efficacy tests. The phenol resistance assay ~~is~~was a component of the AOAC Use-Dilution Test methods, as well as the Tuberculocidal Activity of Disinfectants method.

(3) Surrogate microorganisms. The Agency has approved the use of several surrogate organisms to be used as replacements for microorganisms that cannot be tested because of biohazards or unavailability of scientifically accepted methods. Applicants should consult with the Agency for guidance on additional surrogates. Examples of surrogate organisms are as follows in paragraphs (f)(3)(i) through (f)(3)(iv) of this guideline. (Ref. 24)

(i) *Mycobacterium bovis* BCG has been adopted as a surrogate for human *Mycobacterium tuberculosis*. See Guidance 810.2200.

(ii) ~~The d~~Duck hepatitis B virus ~~test~~ (DHBV) has been adopted as a surrogate for the chimpanzee test used in testing efficacy of disinfectants against human hepatitis B virus (Ref. 6). The data consistency control (method validation) stated in the referenced protocol utilizing two dilutions of BTC 835 has been eliminated.

(iii) ~~The b~~Bovine viral diarrhea virus (BVDV) has been adopted as a surrogate for the hepatitis C virus (Ref. 7). The data consistency control (method validation) stated in the referenced protocol utilizing two dilutions of BARDAC 2280 has been eliminated.

(iv) ~~The f~~Feline calicivirus has been adopted as a surrogate for the Noroviruses (Ref.8). The data consistency control (method validation) stated in the referenced protocol utilizing two dilutions of Bleach has been eliminated.

(4) Antimicrobial rinses for fruits and vegetables. To support label claims for consumer-use products as antimicrobial rinses for fruits and vegetables, products must ~~should~~ meet a two log reduction of five outbreak strains of *Salmonella spp.*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. Currently there is no standard method for assessing the efficacy of antimicrobial rinses for pathogen reduction on the surfaces of fruits and vegetables (raw

agricultural commodities). Applicants should consult with the Agency prior to conducting testing to support this use.

(5) Use of Dacron Loops. The AOAC International has accepted the use of Dacron loops (also termed braided polyester), instead of silk suture loops, for peracid based chemistry ~~peracetic acid~~ containing products, as a method modification to the AOAC Sporicidal Activity Test. Consult the Agency for other chemistries. (Ref. 9).

(g) Special situations. When it is intended that an antimicrobial product be used in a manner that is not reflected by the test conditions specified in the recommended AOAC methods (e.g., inclusion of organic soil or hard water), one or more test conditions specified in the method should be modified and/or supplementary data developed in order to provide meaningful results relative to the conditions of use of the product. The information in paragraphs (g)(1) through (g)(4) in this guideline is critical to the development and submission of the appropriate supportive efficacy data.

(1) Type of surface. When an antimicrobial product is intended to be effective in treating a hard, porous surface, some of the recommended methods may be modified to simulate this more stringent condition by substitution of a hard, porous surface carrier (e.g., porcelain penicylinder or unglazed ceramic tile) for the hard, nonporous surface carrier (stainless steel cylinder or glass slide) specified in the method. In addition, control data (e.g., quantitation of dried carrier, neutralization confirmation, sterility controls) must be developed to assure the validity of the test results when this modification of the method is employed. Since the use of a hard, porous surface would simulate the more stringent test condition, demonstrated efficacy on hard, porous surfaces would ~~generally~~ suffice to support an analogous claim for efficacy of the product on hard, non-porous surfaces as well.

(2) Hard water claim. Any product that bears label claims for effectiveness in hard water must be tested by the appropriate method which has been modified to demonstrate effectiveness of the product in synthetic hard water at the level claimed. The hard water tolerance level may differ with the level of antimicrobial activity claimed (e.g., sterilization, disinfection, or sanitization). To establish ~~disinfectant~~ efficacy in hard water, all microorganisms (bacteria, viruses, and fungi) claimed to be controlled by the product must be tested by the appropriate recommended method at the same hard water tolerance level. Refer to the AOAC International Germicidal and Detergent Sanitizing Action of Disinfectants test (Official Method 960.09) for guidance on the preparation of synthetic hard water (Ref. 10).

(3) Organic burden.

(i) An antimicrobial substance identified as a one-step cleaner-disinfectant or cleaner-sanitizer, or intended to be effective in the presence of light to moderate amounts of organic burden must be tested for efficacy by the appropriate methods which have been modified to include a minimum of a 5% representative organic soil such as blood serum or scientifically accepted equivalent as serum may be inhibitory to some viruses (Ref. 11). Registrants should check with the Agency to determine the acceptability of an organic burden other than blood

serum.

(ii) A suggested procedure to simulate in-use conditions where the antimicrobial agent is intended to treat dry inanimate surfaces contaminated with an organic soil load involves contamination of the appropriate carrier surface with each test microorganisms culture containing 5% v/v blood serum (e.g., 19 mL test microorganism culture +1 mL blood serum) prior to the specified carrier-drying step in the method. Additional organic material need not be incorporated into those procedures where at least 5 percent blood serum is already present in the microbial inoculum to be dried on the surface. Control data (e.g., quantitation of dried carrier counts, neutralization confirmation, sterility controls) must also be developed to assure the validity of the test results when this modification is incorporated into the method. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. A cleaning step must be recommended on the label prior to the application of the antimicrobial agent when the surface to be treated has heavy soil deposits.~~;~~

~~The effectiveness of antimicrobial agents should be demonstrated in the presence of a specified soil at an appropriate concentrated level when specifically claimed and/or indicated by the pattern of use.~~

(iii) A suggested procedure for incorporating a light to moderate organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC International Fungicidal Activity of Disinfectants test (Ref. 12) and the Quantitative Tuberculocidal Test (Ref. 13) involves adding a minimum of 5% (v/v) blood serum directly to the test organism (e.g., 4.75 mL test organism + 0.25 mL blood serum).

~~(iv) When a product is recommended for certain patterns of use where the organic soil claimed is of a specific type, such as soap film residue, the product should be tested in the presence of that specific organic soil. Registrants should provide specific information in the data report regarding the way in which the organic soil, such as soap film residue was prepared (e.g., percentages of ingredients).~~

(4) Exposure period. The exposure period required for an antimicrobial product to be effective may be shorter than the exposure period specified in the recommended method. A modification to provide a shorter exposure period is restricted by the manipulative limitations inherent in the test procedure. A modification to provide a longer exposure period is restricted by the use patterns (e.g., an exposure period of >10 min cannot be recommended for a product that will effectively evaporate from the treated surface in ≤10 min) of the product. If the product is to be represented in labeling for use at exposure periods shorter than those specified in the method, the method should be modified appropriately. ~~in a manner acceptable to the Agency, to reflect the deviation in exposure intended. For liquid products containing volatile active ingredients where the product is applied to an environmental surface, the exposure period should be determined by the AOAC International Germicidal Spray Products As Disinfectants test (Ref. 14). Use of methods that immerse contaminated carriers in the disinfectant fluid would not closely simulate the way in which the volatile disinfectants perform on environmental surfaces.~~

(5) **Use / Reuse of product.** Standardized laboratory tests (such as the AOAC methods) are designed for determining the efficacy of freshly prepared antimicrobial solutions intended for a single application. The more stringent claim of efficacy for a reuse product solution is an optional pattern of use that requires a specifically designed protocol to demonstrate retention of the claimed levels of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges for the number of days or applications specified in the directions for use. Any registrant interested in conducting the optional reuse testing is expected to contact the Efficacy Evaluation and Technical Management Section of the Antimicrobial Division for specific information and guidance for developing an appropriate detailed reuse protocol relative to label efficacy claims and patterns of use intended for the reuse product solution. Each simulated use protocol for reuse testing must include the following minimal specifications to assure that a minimally stressed used solution is employed in determining antimicrobial efficacy:

(i) A simulated use procedure is performed with two samples of the product, representing two different batches.

(ii) A complete set of inhalation equipment (two sections of corrugated rubber tubing, each 3 to 4 feet in length, one 2-3 liter rebreathing bag, one face mask, one endotracheal tube, one Y connector) is used for each 2.5 gallons of test solution (e.g., two sets for 5 gallons by manual methods and six sets for 15 gallons in automatic machine processing.)

(iii) Each set of equipment is subjected to a treatment cycle consisting minimally of cleaning with a detergent, rinsing with water, and soaking of the test equipment in the test solution as follows:

(A) Five cycles per day in an automatic disinfecting machine.

(B) Three cycles per day by manual methods for disinfecting and sterilizing.

(C) One cycle per day by manual methods for sterilizing only.

(iv) The product must be stressed periodically by adding microbial bioburden directly to the test solution. The bioburden consisting of dried carriers of *Staphylococcus aureus, Salmonella enterica,* and *Pseudomonas aeruginosa* when the product is used as a disinfectant, and spores of *Bacillus subtilis* and *Clostridium sporogenes* when the product is intended for use as a sterilant. Each carrier must possess a recoverable titer of 10^4 organisms. The minimum cumulative bioburden, added to the test solution by either Option I or Option II (under paragraph (c)(4)(v)(D)(1) or (c)(4)(v)(D)(2) of this guideline) is approximately two bacterial contaminated carriers per 100 mL of use solution which provides a stress of at least 2×10^4 carrier-dried viable microorganisms per 100 mL of test solution per day).

(A) Option I: The daily deliberate bacterial contamination is added directly to the total volume of product solution in the container. For example: 900 contaminated carriers of one of the three specified test microorganisms would be added daily to the 15 gallons of use solution in the reservoir of an automatic disinfecting machine. A total of 12,600 contaminated carriers

would be required for 70 cycles run during a 14-day use period.

(B) Option II: The daily deliberate bacterial contamination is added to an aliquot (1 Liter) removed from the total volume of product solution in the container. After soaking, the contaminated use solution is returned to the container except at the last cycle in the study when the contaminated use solution is tested for antimicrobial activity. For example: 180 contaminated carriers of one of the three specified test microorganisms would be added daily to 1 Liter of use solution removed from the reservoir of an automatic disinfecting machine to provide a bioburden load to the use solution in the reservoir. A total of 2,520 contaminated carriers would be required for 70 cycles during a 14-day use period.

(C) The addition of bioburden by the Option II procedure is simpler and not as costly to perform as Option I. The total cumulative bioburden load in the stressed solution by either option is approximately 0.02 carriers per mL or 2 carriers per 100 mL as determined by the equation:

$$K = Bc/(Tr)(Vt)$$

where:

K = a constant approaching 0.02
B = bioburden (number of contaminated carriers)
T = Time for reuse (days)
V = Volume (mL)

The determined total cumulative bioburden by this equation for each example option is given by:

Option I:

$$K = 12{,}600 / 14 \times 56{,}775 = 0.016$$

Option II:

$$K = 2{,}340 / 13 \times 56{,}775 + 180 / 14 \times 1{,}000 = 0.003 + 0.013 = 0.016$$

Either option for adding the bioburden will provide an equivalent bioburden load to the terminal stressed used solution that is tested for antimicrobial activity. In Option I, a large bioburden load is added daily to the total solution in the reservoir throughout the test period. In Option II, most of the bioburden load is added specifically to the volume of used solution removed from the container at the end of the test period with only a token bioburden load added daily to the total solution in the reservoir.

(v) The concentration of the active ingredients and the pH of the solution is determined during the simulated use study.

(vi) For further assurance that the used solution will be effective under actual use conditions, the reuse protocol may add other factors to stress the used solution. An acceptable, but optional, procedure is the addition of 2% blood serum to the fresh solution at the start of the test to simulate the protein that may be present on items that are difficult to thoroughly clean. Hard water may also be incorporated into the test procedure.

(vii) After the completion of the simulated use study for the intended number of days, the stressed solution is tested by specified AOAC and other EPA-recommended laboratory test methods to support efficacy of the reused solution.

(viii) Only data derived from protocols conducted under the above conditions will be considered in support of efficacy claims for the used solution even though it may be possible to demonstrate a longer reuse period with different equipment/instruments under some other prevailing conditions of use.

(6) Confirmatory testing. In certain situations an applicant may rely on previously submitted efficacy data to support an application or amendment for registration of a product and submit only confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formulation. These situations are as listed in paragraphs (C)(3)(i) through (C)(3)(iii) of this guideline:

(i) Duplicated Product Formulations. In this situation, the applicant manufactures a formulation which duplicates a product that is already registered with complete supporting efficacy data. The chemical composition, manufacturing procedure, label claims, and directions for use are identical in substance to those of the original registration, and specific references (Master Record ID Numbers [MRIDs]) to the supporting data developed for the original product are cited by the applicant.

(ii) Minor Formulation Change in a Registered Product. In this situation, the change in the formulation is relatively minor, e.g., a change of an inert ingredient. The label claims and directions for use are unchanged from those accepted for the registered formulation, and specific references (MRIDs) to the supporting data developed for the original formulation are cited by the applicant. If the only change in the formulation is the addition of a fragrance or dye, confirmatory data does not need to be submitted. However, when the product is an aerosol formulation, confirmatory data should be submitted for all formulation changes, including the addition of fragrances and dyes.

(iii) The confirmatory data must be developed on the applicant's own finished product. When the test methodology utilized in deriving the original supporting efficacy data were modified to include additional elements not specified in the recommended method, such as organic soil, hard water, longer or shorter contact time, etc., the confirmatory data should be produced under similarly modified conditions.

(h) Microbiological technique considerations

(1) Microorganism survival after drying on a hard surface. Quantitative determinations of the microbial counts on the untreated control carriers after drying must be conducted in order to determine the validity of the test results obtained with the treated carriers. The control carrier count evaluation must be performed at the completion of the test. These

quantitative determinations must be performed for all carrier-based assays, whether or not modifications are made to the method being used. The test results must include the individual or pooled dried carrier counts obtained by the method. The detailed final report for this testing should include information and descriptions regarding: preparation of the inoculum; application of the inoculum to the carrier; the time/temperature and relative humidity conditions for drying the microorganisms on the carrier; the technique for removal of the microorganisms from the carrier; and the specific assay procedure indicating such details as replication, subculture media, diluents, and the incubation time/temperature conditions for the enumeration procedure employed.

(2) Microorganism survival for suspension tests. Quantitative determination of the microbial count of the inoculum in a parallel untreated diluent must be conducted in order to determine the level of microbial challenge in the test (Numbers Control). These quantitative determinations must be performed for all suspension assays, whether or not modifications are made to the method being used. The test results must include the individual counts obtained by the recovery method. The detailed final report for suspension testing must include information and descriptions regarding: preparation of the inoculum, the volumes used for inoculation, and the specific assay procedure indicating such details as replication, subculture media, diluents, and the incubation time/temperature conditions for the enumeration procedure employed.

(3) **Neutralization.** Neutralization is the a process for inactivating or quenching antimicrobial activity during efficacy testing. This may be achieved through physical (e.g. filtration, dilution, secondary subculture) and/or chemical (e.g., addition of sodium thiosulfate to the diluent) means. For each efficacy test, neutralization procedures ~~should~~ must be employed, at the completion of the contact time, in order to preclude residual effects of the active ingredients in the subculture medium. If neutralization is not properly employed, the results of efficacy testing may be exaggerated. ~~A specific medium capable of neutralizing the antimicrobial effects of a product should be employed prior to the microbiological assay. In addition, data should be submitted to demonstrate that the neutralizer employed inactivates the active ingredients and does not possess any antimicrobial activity itself. Some of the recommended methods described in this section rely solely upon the selection of an appropriate subculture medium to neutralize the antimicrobial effects of certain general types of chemical compounds (active ingredients). In lieu of chemical neutralization, it should be documented that appropriate subculture techniques have been employed that preclude residual carryover of active ingredients. To document the absence of residual effects of the active ingredients in the subculture medium, the procedures in paragraphs (h)(3)(i) through (h)(3)(ii)(C) in this guideline should be followed.~~ The method of neutralization, whether that be physical or chemical, must be confirmed. Data must be submitted (Neutralization Confirmation) in the final report to demonstrate that the neutralization method sufficiently inactivated the active ingredient(s) and that any neutralizer medium used did not possess any antimicrobial activity. (Ref. 18)

(i) ~~Efficacy final reports should describe the neutralization techniques employed during the study. In addition, evidence should be submitted to demonstrate that the neutralizer identified inactivates the antimicrobial ingredient and that the neutralization process itself does not possess any antimicrobial activity. These controls are termed Neutralization Confirmation.~~

~~This confirmation is conducted by demonstrating the growth of an inoculum of 5-100 CFU test organism/mL growth media into a parallel test (including the neutralization process) conducted without the test organism or following incubation of the actual test. In addition, the Neutralization Confirmation inoculums is inoculated into the parallel test without the neutralization process to confirm lack of antimicrobial activity of the process (Ref. 15).~~
Examples of neutralization techniques:

(A) In ~~carrier-based~~qualitative test methods, after treatment, the inoculated carrier is initially deposited in a tube of growth media (i.e., primary subculture). If the primary subculture tube is not sufficient to achieve neutralization by the use of a chemical agent and/or through dilution of the test substance, t~~T~~he carrier may then be transferred to a second tube of growth media (i.e., secondary subculture). ~~The primary and/or the secondary subculture may include a chemical agent to achieve neutralization~~. Secondary subcultures may be helpful in achieving neutralization. The ~~either through dilution or incorporation of chemical agents in the growth media. A~~ neutralization procedure employed must be confirmed and reported by running a parallel test with uninoculated carriers being added to growth media containing a low level (i.e. 10-100 CFU/ml) of bacteria. ~~confirmation for carrier based test methods may be conducted by demonstrating the growth an inoculum 5-100 CFU of test organism/ml growth media, into a parallel test with the neutralization process conducted without the test organism or following incubation of the actual te~~Both the primary cultures and secondary subcultures ~~should~~ must be incubated and checked for growth in the test and in the neutralization confirmation. Dried test carriers should not be used to test the ability of a subculture medium to support organism growth, as this would provide too large a bioburdern and may lead to an inaccurate evaluation of the presence of any bacteriostasis that may result from the carry-over of the antimicrobial substance on the carrier to the subculture medium. Growth results for both primary and secondary subcultures ~~should~~ must be reported for the test and neutralization confirmation in the final report.

(B) ~~A~~ In quantitative test methods, neutralization confirmation must be conducted for the all ~~neutralization/recovery methods employed in testing for suspension based test methods should be conducted for all neutralization/recovery methods employed in testing.~~ Neutralization confirmations may be conducted by neutralizing the test substance, without the organism, as in the test. Followed by inoculation of a low level of inoculum (e.g. for bacteria 10-100 CFU/ml) ~~or organism (5-100 CFU/ml)~~ and subsequent plating. Plate counts must ~~should~~ be within 1.0 log of a parallel population control. (Ref. 15)

(C) For virucidal tests, scientifically accepted controls, including proper neutralization controls ~~should~~ must be performed (e.g., ASTM E1482) (Ref. 16).

(4) Batch replication for modified tests. Where batch replication has already been performed and accepted for a product registration with unmodified tests by the recommended methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carriers, etc.) may be conducted with reduced batch replications as in paragraphs (h)(4)(i) and (h)(4)(ii) in this

guideline.

(i) For basic efficacy claims (e.g., sterilants, disinfectants, sanitizers), two samples, representing two different batches, instead of three.

(ii) For supplemental efficacy claims (e.g., fungicides, virucides, and tuberculocides), one sample instead of two.

(5) **Validation of efficacy**. The Agency reserves the option to perform its own tests for validation of efficacy of products selected on a case-by-case basis.

(6) **Test failure**. Failure of a test material to meet the specified testing or performance requirements may be reportable under FIFRA section 6(a)(2). For test material not meeting the efficacy requirements for health-related claims, repeat testing is permitted in the following circumstances to eliminate the possibility of false positives as stated in the AOAC Use-Dilution Method (Ref. 4):

(i.) Test results show 2 – 3 positives / 60 carriers tested

(ii) The growth reported following testing is confirmed to be a contaminant and is not the test organism.

(iii) An operator error occurred during testing of a given batch (e.g. penicylinder touched side of treatment tube, dropped carrier)

(i) Data collection and reporting

(1) General. 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 a minimum:

(i) Study title;

(ii) Product identity;

(iii) Guideline number;

(iv) Identification of the testing laboratory or organization;

(v) Location where the test was performed;

(vi) Name(s) of the person(s) responsible for the test;

(vii) Good Laboratory Practice compliance;

(viii) Purpose of the study;

(ix) Date of the start and end of the test;

(x) Statistical treatment of the data;

(xi) Conclusions;

(xii) References;

(xiii) Appendices including study protocol;

The applicant is encouraged to use the EPA's standard efficacy report format, which may be found at http://www.epa.gov/oppad001/efficacystudystandards.htm

(2) Data for recommended methods. When recommended methods from the Official Methods of Analysis of AOAC International; the Annual Book of Standards of the American Society for Testing and Materials; or, EPA methods are used to develop efficacy data, certain minimal information, in addition to that described in this guideline, should be provided in the test report. The report should include, at a minimum:

(i) Test employed, and any modifications thereto (e.g., organic soil, hard water, etc);

(ii) Test microorganisms employed, including identification of the specific strain (ATCC or other);

(iii) Description of the test substance, including the percent of active ingredient;

(iv) Concentration or dilution of the product tested and how prepared;

(v) Number of samples, batches and replicates tested;

(vi) Preparation dates of each product batch (individually formulated preparation of the product);

(vii) Identification of all material or procedural options employed, where such choice is permitted or recommended in the test method selected (e.g., growth media, drying time for inoculated carriers, neutralization confirmation and/or subculture media, secondary subculturing);

(viii) Test exposure conditions (e.g., contact time, temperature, and relative humidity);

(ix) Complete reports of results obtained for each replication;

(x) Any control data essential to establish the validity of the test (e.g. neutralization confirmation);

(xi) Carrier counts or numbers control for suspension tests;

(xii) Any additional data pertinent for specific tests described in this guideline.

(3) Data for modifications of recommended methods. Where recommended methods are modified to support specific claims and/or use patterns for a product, the protocol, identifying and describing each modification, ~~should~~ must be provided in specific detail with the test report. The applicant is encouraged to submit the proposed modification to the Agency for review and evaluation prior to initiation of the test.

(4) Data for other methods. When recommended methods, or modifications thereto, are not employed to develop efficacy data (such as actual in-use or many kinds of simulated-use testing), complete testing protocols should be submitted with the test reports. All materials and procedures employed in testing should be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should be provided with the test reports. The applicant should submit the proposed testing protocols for in-use or simulated-use studies to the Agency for review and evaluation prior to initiation of the test.

(j) References. The references in this paragraph may be consulted for additional background information:

(1) Environmental Protection Agency, Pesticide Registration Notice ~~PR~~–2000-1, Applicability of the Treated Articles Exemption to Antimicrobial Pesticides, March 6, 2000. Office of Pesticide Programs, Antimicrobials Division. See http://www.epa.gov/PR_Notices/.

(2) Environmental Protection Agency, Pesticide Registration Notice PR 91-2, Accuracy of Stated Percentages for Ingredients Statement, May 2, 1991. Office of Pesticide Programs, Antimicrobials Division. See http://www.epa.gov/PR_Notices/.

(3) *Official Methods of Analysis of AOAC International*. Chapter 6, Disinfectants, Hard Surface Carrier Test Methods, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(4) *Official Methods of Analysis of AOAC International*. Chapter 6, Disinfectants, Use-Dilution Methods, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(5) Environmental Protection Agency, Pesticide Registration Notice 2001-4, Elimination of Phenol Resistance Testing for Antimicrobial Disinfectant and Sanitizer Pesticides. Office of Pesticide Programs, Antimicrobials Division. See http://www.epa.gov/PR_Notices/.

(6) Protocols for Testing the Efficacy of Disinfectants Against Hepatitis B Virus (HBV).

Office of Pesticide Programs, Antimicrobials Division. See http://www.epa.gov/oppad001/regpolicy.htm.

(7) Virucidal Effectiveness Test Using Bovine Viral Diarrhea Virus (BVDV) as a Surrogate for Human Hepatitis C Virus. Office of Pesticide Programs, Antimicrobials Division. See http://www.epa.gov/oppad001/regpolicy.htm.

(8) Virucidal Effectiveness Test Using Feline Calicivirus as a Surrogate for Norovirus. Office of Pesticide Programs, Antimicrobials Division. See http://www.epa.gov/oppad001/regpolicy.htm.

(9) McDonnell, G. (2003) *J. AOAC Int.* 86,407-411.

(10) *Official Methods of Analysis of AOAC International.* Chapter 6, Disinfectants, Official Method 960.09 - Germicidal and Detergent Sanitizing Action of Disinfectants, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(11) *Annual Book of ASTM Standards*, Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces, Designation E1053~~-97~~. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428~~, current edition.~~ .

(12) *Official Methods of Analysis of* AOAC *International.* Chapter 6, Disinfectants, Official Method 955.17 Fungicidal Activity of Disinfectants. Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(13) Environmental Protection Agency, Data Call-in Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides with Tuberculocidal Claims (Registration Division, Office of Pesticide Programs, June 13, 1986). See http://www.epa.gov/oppad001/dis_tss_docs/dis-06.htm.

(14) *Official Methods of Analysis of AOAC International.* Chapter 6, Disinfectants, Official Method 961.02 - Germicidal Spray Products as Disinfectants, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(15) *Annual Book of ASTM Standards*, Standard Test Methods, Evaluation of Inactivators of Antimicrobial Agents, Designation E1054~~-02~~. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428~~, current edition~~.

(16) *Annual Book of ASTM Standards*, Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations, Designation E1482~~-04~~. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428~~, current edition~~

(17) General Requirements for Antimicrobial Agents (Pesticide Assessment Guidelines,

Subdivision G, Product Performance, EPA report 540/9-82-026, October 1982).

~~(17)~~(18) Antimicrobial Science Policies; Disinfectant Technical Science Section (DIS/TIS); http://www.epa.gov/oppad001/sciencepolicy.htm. Note: website does not include sterilant, Use/ Re-Use, Towelette or In-tank sanititizer DIS/TSS documents.

~~(18)~~(19) ASTM E2562; Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor.

~~(19)~~(20) ASTM E 2196; Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with Shear and Continuous Flow using a Rotating Disk Reactor.

~~(20)~~(21) ASTM E1839; Standard Test Method for Efficacy of Slimicides for the Paper Industry – Bacterial and Fungal Slime.

~~(21)~~(22) US EPA Re-use Test Protocol Specifications

(23) NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Sixth Edition. NCCLS document M7-A6 (ISBN 1-56238-486-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne Pennsylvania 19087-1898 USA, 2003.

(24) Pesticides: Regulating Pesticides; Implementation of the Emerging Pathogens and Disinfection Hierarchy for Antimicrobial Products; April 3, 2008; http://www.epa.gov/oppad001/disinfection_hier.htm

CSPA Comments on 810.2100
6/28/2010

OPPTS 810.2100: Sterilants—efficacy data recommendations for public health uses

(a) Scope

(1) Applicability. This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7U.S.C. 136, et seq.) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 346a). It addresses testing to demonstrate effectiveness of products which are sterilants.

(2) Background. The source materials used in developing this OPPTS test guideline are OPP guidelines 91-2: Products for use on hard surfaces and 91-30: Acceptable methods (Pesticide Assessment Guidelines, Subdivision G, Product Performance. EPA report 540/9-82-026, October 1982.

(b) Purpose. This guideline addresses efficacy testing for antimicrobial pesticides intended to be used on hard, inanimate, environmental surfaces, and, which bear label claims as sterilants.

(c) General considerations

(1) This guideline recommends tests to be conducted and data to be submitted which the Agency believes will generally satisfy the requirements for pesticide registration. Studies conducted under this guideline should be completed under EPA's Good Laboratory Practice regulations (40 CFR Part 160). Note: The Association of Official Analytical Chemicals (AOAC) recommended tests are expected to be conducted as written. For deviations (e.g., cultures grown with shaking instead of static, dilution of culture prior to drying on carriers) proposed to be used in the conduct of these tests, obtain written approval from the Agency and document such deviations in the study reports submitted to the Agency. Refer to OPPTS Test Guideline 810.2000 for general testing recommendations prior to initiating tests.

(2) Validation testing approaches, which may be needed to augment the full range of efficacy tests in special circumstances, are also described.

(d) Water-soluble powders and ~~non-volatile~~ liquid products

(1) Test procedure. The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method 1 (Ref. 1) to demonstrate the sterilant efficacy of products. For *Bacillus subtilis* Method II may be used. Sixty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) should be tested against spores of both *Bacillus subtilis* (*B. subtilis*) (American Type Culture Collection (ATCC) 19659) and *Clostridium sporogenes* (C. *sporogenes*) (ATCC 3584) on three samples representing three different batches of the product, one of which should be ~~at least~~ ≥ 60 days old (240 carriers per sample, or a total of 720 carriers). ~~The inoculum~~

~~employed should provide a count of x 10^5 — 1 x 10^6 colony forming units per carrier.~~ Any sterilant which is a vapor or gas and is recommended for use in a specific device should be tested using the AOAC International Sporicidal Activity of Disinfectants test in that specific device and according to the directions for use of that specific device. Modifications to the AOAC Sporicidal Activity of Disinfectants test to address this use should be submitted to the Agency for review and approval prior to conducting the tests.

(2) Evaluation of sterilant success. The product should kill the test spores on all of the 720 carriers without any failures (e.g. growth of test organism after carrier treatment).

(e) Validation testing for all products with sterilant claims. Data submitted to support sterilant claims are subject to independent validation testing in a second laboratory or can be tested in the same lab with separate staff.

(1) Test procedure. The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method I (Ref. 1) to demonstrate the sterilant efficacy of products. For *Bacillus subtilis* Method II may be used. Thirty carriers representing each of the two types of surfaces (porcelain penicylinders and silk suture loops), should be tested against the spores of both *B. subtilis* and *C. sporogenes* on one sample of the product. ~~The inoculum employed should provide a count of x 10^5 — 1 x 10^6 colony forming units per carrier.~~

(2) Evaluation of sterilant success. The product should kill the test spores on all 120 carriers without any failures (e.g. growth of test organism after carrier treatment).

(f) Sprays, gases, and foams. (Reserved.)

(g) Additional spore formers, *Clostridium difficile* (*C. difficile*) claims. This section addresses guidance for products with claims to inactivate *C. difficile* spores on hard, non-porous, inanimate surfaces. The Agency recommends three possible options, as described in paragraphs (g)(1)(i) through (g)(1)(iii) of this guideline. The validation testing described in section (e) (2) and (e) is not required for *C. difficile* spore claims.

(1) Water-soluble powders and liquid products, qualitative testing

(i) Test procedure for sterilant/sporicide plus *C. difficile* claim. For the sterilant claim, the Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, ~~Method II (Ref. 1)~~ Method I (Ref. 1) ~~to demonstrate the sterilant efficacy of products~~ For *Bacillus subtilis* Method II may be used as described in (d)(1). In addition, conduct a **confirmatory test** using the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, ~~Method I~~ modified for *C. difficile* testing. Until the Agency identifies a representative toxigenic strain or suitable surrogate(s) to be used in testing against *C. difficile*, one of the following toxigenic strains should be used for testing: ATCC 700792, ATCC 43598 or ATCC 43599. *C. difficile* spores are inoculated on thirty carriers (porcelain penicylinders) for two

samples, representing two different batches of the product (a total of 60 carriers).

(A) Evaluation of sporicidal success. The product should kill all of the test spores on all of the initial and confirmatory carriers (780 carriers) without any failures (e.g. growth of test organism after carrier treatment).

(B) Reserved.

(ii) Test procedure for *C. difficile* sporicides--qualitative testing. The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, ~~Method I~~ (Ref. 1) modified for *C. difficile* testing using *C. difficile* ATCC 700792, ATCC 43598 or ATCC 43599. Sixty carriers (porcelain penicylinders) should be tested on three samples representing three different batches of product, one of which should be ≥ 60 days old (a total of 180 carriers). ~~The inoculum employed should provide a count of $x \cdot 10^5$ — 1×10^6 colony forming units per carrier.~~

(A) Evaluation of sporicidal success. The product should kill all of the test spores on all of the 180 carriers without any failures (e.g. growth of test organism after carrier treatment).

(B) Reserved.

(iii) Test procedure for *C. difficile* sporicides--quantitative testing. The Agency recommends ~~the~~ use of the AOAC Method 2008.05: Quantitative Three Step Method (TSM) (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface) (Ref. 2) or ASTM E 2197: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides (Ref. 3). Until the Agency identifies a representative toxigenic strain or suitable surrogate(s) to be used in testing against *C. difficile*, one of the following toxigenic strains should be used for testing: ATCC 700792, ATCC 43598 or ATCC 43599. ~~The inoculum employed should provide a target count of ≥ 10^6 colony forming units per carrier.~~ The product should be tested on three samples representing three different batches of product, one of which should be ≥60 days old. For the AOAC TSM use 10 carriers per lot. For the ASTM E 2197, use 10 carriers per test lot and 3 carriers for the control.

(A) Evaluation of sporicidal success. The product should achieve a log reduction of ~~at least~~ ≥ 6 logs based on recoverable spores.

(h) Sprays, gases, and foams. (Reserved.)

(i) *Bacillus anthracis* (*B. anthracis*) claims. This section addresses efficacy tests for all products with claims to inactivate *B. anthracis* spores on inanimate surfaces. The Agency recommends three possible approaches, as described in paragraphs (h)(1)(i) through (h)(1)(iii) of this guideline.

(1) Water-soluble powders, liquid products, gases and vapors—(i) Test procedure for sterilant/sporicide plus *B. anthracis* claim. The Agency recommends use of the Official

Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test (Ref. 1) to demonstrate the sterilant efficacy of products. Sixty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) should be tested against spores of both *B. subtilis* (ATCC 19659) and C. *sporogenes* (ATCC 3584) on three samples representing three different batches of the product, one of which should be ~~at least~~ ≥ 60 days old (240 carriers per sample, or a total of 720 carriers). ~~The inoculum employed should provide a target count of 1×10^5 — approximately 1×10^6 colony forming units per carrier.~~ In addition, conduct a **confirmatory test** using virulent *B. anthracis* spores (or a surrogate acceptable to EPA; e.g. *B subtilis* ATCC 19659, inoculated on thirty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) on two samples, representing two different batches of the product (a total of 120 carriers).

(A) Evaluation of sporicidal success. The product should kill all of the test spores on all of the initial and confirmatory carriers (840 carriers) without any failures (e.g. growth of test organism after carrier treatment).

(B) Reserved.

(ii) Test procedure for sporicidal decontaminants--qualitative testing. The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test (Ref. 1) using virulent *B. anthracis* spores (or a surrogate acceptable to EPA). Sixty carriers representing either or both of two types of surfaces (porcelain penicylinders and/or silk suture loops) should be tested on three samples representing three different batches of product, one of which should be ~~at least~~ ≥60 days old. ~~The inoculum employed should provide a count of $x \times 10^5$ — 1×10^6 colony forming units per carrier.~~ If one surface type is tested, then there are 60 carriers per sample, or a total of 180 carriers; if both surfaces types are tested, then the total number of carriers is 360.

(A) Evaluation of sporicidal success. The product should kill all of the test spores on all of the 180 (or 360) carriers without any failures.

(B) Reserved.

(iii) Test procedure for sporicidal decontaminants--quantitative testing. The Agency recommends the use of a well developed, quantitative sporicidal test method acceptable to EPA using virulent *B. anthracis* spores (e.g. *B. subtilis* ATCC 19659) on porous and/or nonporous surfaces acceptable to EPA. ~~The inoculum employed should provide a target count of approximately ≥1×10^7 colony forming units per carrier.~~ The product should be tested on three samples representing three different batches of product, one of which should be ≥60 days old. The number of carriers will vary depending on the test method. The coupon material(s) should be representative of those found at the site(s) that appear on the product's labeling, and be acceptable to EPA.

(A) Evaluation of sporicidal success. The product should achieve a log reduction of ≥6 logs based on recoverable spores.

(B) Reserved.

(2) Simulated use testing for gas and vapor products—(i) Test procedure. In addition to conducting one of the three laboratory studies in paragraphs (k)(1)(i) through (h)(1)(iii) of this guideline, simulated-use testing should also be conducted for vapor and gas products. Protocols for the simulated-use test should be submitted to the Agency for review and approval prior to conducting the test. The testing should be conducted under conditions that are representative of the uses specified on the product's labeling, and in a setting that is representative of the label use site(s). For example, a product intended for use in a room or a large warehouse should be tested in an empty room or large chamber. The purpose of the test would be to assure that key parameters for efficacy (chemical concentration, temperature, relative humidity and contact time) are accurately monitored and maintained throughout the enclosed space, and establish product generation rate (lbs/hr) and rate/volume (lbs/hr/ft3).

(ii) Additional considerations. Important issues to consider in developing the protocol for this test include:

(A) The test should be ~~set-up~~conducted in a sealed enclosure at least the size of a typical office or other room t~~h~~at simulates the intended use pattern ~~intended~~ and designed to measure the distribution of the product and conditions needed to meet the measure of success in the laboratory efficacy test. Items that might be treated (e.g., dressers, upholstered furniture, carpet, etc.) during an actual fumigation, should be included in this test.

(B) The protocol should specify the dimensions of the enclosure, number and location of monitoring devices (e.g., for gas or vapor concentration, total mass of gas or vapor injected into the enclosure, temperature, relative humidity), product application equipment, heaters and fans, contact time, etc. The equipment used to monitor and maintain these test parameters should be described.

(C) All recorded test results pertaining to the test conditions/parameters should be submitted to the Agency. The maximum volume of space that can be treated with a particular unit should be reported to the Agency. The minimum total mass of gas or vapor required to maintain the required concentration and contact time per cubic foot of space to be decontaminated should be reported.

(D) Appropriate positive and negative controls should be employed.

(E) This test must be conducted either in accordance with Good Laboratory Practices (GLP) per 40 CFR Part 160 or in a federal laboratory with an appropriate Quality Assurance Project Plan (QAPP).

(iii) Evaluation of sporicidal success. Measurements should show that the same concentration, temperature, and relative humidity, can be maintained for the required contact time needed to achieve 100% kill (i.e., no growth of the test organism on any of the carriers) in the qualitative laboratory test, or a 6 log reduction in the quantitative test is demonstrated in the simulated-use test. In addition, measurements of the fumigant mass injection/generation rate

(e.g., pounds/hour), divided by the volume of the simulated use test bed, that was used to arrive at the required generation rate/volume (e.g., pounds per hour/cubic foot) for the fumigation, should be included with the data, and listed on the product label.

~~**(j) Data collection and reporting**~~

~~**(1) General.** 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 a minimum:~~

~~**(i)** Study title;~~

~~**(ii)** Product identity;~~

~~**(iii)** Guideline number;~~

~~**(iv)** Identification of the testing laboratory or organization;~~

~~**(v)** Location where the test was performed;~~

~~**(vi)** Name(s) of the person(s) responsible for the test;~~

~~**(vii)** Good Laboratory Practice compliance;~~

~~**(viii)** Purpose of the study;~~

~~**(ix)** Date of the start and end of the test;~~

~~**(x)** Statistical treatment of the data;~~

~~**(xi)** Conclusions;~~

~~**(xii)** References;~~

~~**(xiii)** Appendices including study protocol;~~

~~The applicant is encouraged to use the EPA's standard efficacy report format, which may be found at http://www.epa.gov/oppad001/efficacystudystandards.htm~~

~~**(2) Data for recommended methods.** When recommended methods from the Official Methods of Analysis of AOAC International; the Annual Book of Standards of the American Society for Testing and Materials; or, EPA methods are used to develop efficacy data, certain minimal information, in addition to that described in this guideline, should be provided in the test report. The report should include, at a minimum:~~

~~**(i)** Test employed, and any modifications thereto (e.g., organic soil, hard water, etc);~~

~~(ii) Test microorganisms employed, including identification of the specific strain (ATCC or other);~~

~~(iii) Description of the test substance, including the percent of active ingredient;~~

~~(iv) Concentration or dilution of the product tested and how prepared;~~

~~(v) Number of samples, batches and replicates tested;~~

~~(vi) Preparation dates of each product batch (individually formulated preparation of the product);~~

~~(vii) Identification of all material or procedural options employed, where such choice is permitted or recommended in the test method selected (e.g., growth media, drying time for inoculated carriers, neutralization confirmation and/or subculture media, secondary subculturing);~~

~~(viii) Test exposure conditions (e.g., contact time, temperature, and relative humidity);~~

~~(ix) Complete reports of results obtained for each replication;~~

~~(x) Any control data essential to establish the validity of the test (e.g. neutralization confirmation);~~

~~(xi) Carrier counts or numbers control for suspension tests;~~

~~(xii) Any additional data pertinent for specific tests described in this guideline.~~

~~**(3) Data for modifications of recommended methods.** Where recommended methods are modified to support specific claims and/or use patterns for a product, the protocol, identifying and describing each modification, must be provided in specific detail with the test report. The applicant is encouraged to submit the proposed modification to the Agency for review and evaluation prior to initiation of the test.~~

~~**(4) Data for other methods.** When recommended methods, or modifications thereto, are not employed to develop efficacy data (such as actual in-use or many kinds of simulated-use testing), complete testing protocols should be submitted with the test reports. All materials and procedures employed in testing should be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should be provided with the test reports. The applicant should submit the proposed testing protocols for in-use or simulated-use studies to the Agency for review and evaluation prior to initiation of the test.~~

(k) References: The references in this paragraph may be consulted for additional background information.

(1) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants,

Official Method 966.04 Sporicidal Activity of Disinfectants, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(2) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 2008.05 Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface), Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(3) *Annual Book of ASTM Standards*, Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides, Designation E 2197-02. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

(4) Guidance for the Efficacy Evaluation of Products with Sporicidal Claims against *Clostridium difficile*; http://www.epa.gov/oppad001/cdif-guidance.html

(5) *Clostridium difficile* product labeling; http://www.epa.gov/oppad001/clostridium_diff.htm

CSPA Comments on 810.2200
6 28 2010

OPPTS 810.2200: Disinfectants for use on hard surfaces - efficacy data recommendations

(a) Scope.

(1) Applicability. This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)(7U.S.C. 136, et seq.) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 I/S/C. 346a). It addresses testing to demonstrate effectiveness of products which are disinfectants, fungicides, virucides, and tuberculocides.

(2) Background. The source materials used in developing this OPPTS test guideline are OPP guidelines 91-2: Products for use on hard surfaces and 91-30: Acceptable methods (Pesticide Assessment Guidelines, Subdivision G, Product Performance. EPA report 540/9-82-026, October 1982).

(b) Purpose. This guideline addresses efficacy testing for antimicrobial pesticides intended to be used on hard surfaces, namely disinfectants, fungicides, virucides, and tuberculocides in a variety of product types (water-soluble powders, liquids, sprays, towelettes, etc.).

(c) General considerations

(1) This guideline specifies which tests are to be conducted and what data is to be submitted which the Agency believes will generally satisfy the requirements for pesticide registration. All studies to be submitted to the Agency for registration which are conducted under this guideline must be completed under EPA's Good Laboratory Practice regulations (40 CFR Part 160). Note: The recommended published test methods; e.g. The Association of Official Analytical Chemists (AOAC) should be followed to ensure compliance while taking into account application of new technologies, alternate product forms, and specific needs for additional organisms. For deviations proposed to be used in the conduct of these tests, ~~obtain written approval from the Agency and~~ document such deviations in the study reports submitted to the Agency. Refer to OPPTS Test Guideline 810.2000 for general testing recommendations prior to initiating tests.

(2) [Reserved]

~~**(3) Confirmatory testing.** In certain situations an applicant may rely on previously submitted efficacy data to support an application or amendment for registration of a product and submit only confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formulation. These situations are as listed in paragraphs (C)(3)(i) through (C)(3)(iii) of this guideline:~~

~~(i) Duplicated Product Formulations. In this situation, the applicant manufactures a formulation which duplicates a product that is already registered with complete supporting efficacy data. The chemical composition, manufacturing procedure, label claims, and directions for use are identical in substance to those of the original registration, and specific references (Master Record ID Numbers [MRIDs]) to the supporting data developed for the original product are cited by the applicant.~~

~~(ii) Minor Formulation Change in a Registered Product. In this situation, the change in the formulation is relatively minor, e.g., a change of an inert ingredient. The label claims and directions for use are unchanged from those accepted for the registered formulation, and specific references (MRIDs) to the supporting data developed for the original formulation are cited by the applicant. If the only change in the formulation is the addition of a fragrance or dye, confirmatory data does not need to be submitted. However, when the product is an aerosol formulation, confirmatory data should be submitted for all formulation changes, including the addition of fragrances and dyes.~~

~~(iii) The confirmatory data must be developed on the applicant's own finished product. When the test methodology utilized in deriving the original supporting efficacy data were modified to include additional elements not specified in the recommended method, such as organic soil, hard water, longer or shorter contact time, etc., the confirmatory data should be produced under similarly modified conditions.~~

(34) Efficacy claims. Table 1 provides a quick reference guide to testing for basic claims described in this guideline. Consult the text for detailed testing descriptions.

Table 1. Testing for basic public health efficacy claims

Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Evaluation of Success
Limited~~disinfectant~~ spectrum products / hard non-porous surfaces	Water soluble powders/liquids or Internal Toilet or Urinal Bowl Surfaces	AOAC Use-Dilution Method or AOAC Hard Surface Carrier Test (distilled water only)	*Staphylococcus aureus* (ATCC 6538) or *Salmonella enterica* (ATCC 10708)	Three batches, one at least 60 days old. 60 carriers against either organism claimed (180 carriers).	59/60 carriers are negative for each batch tested for all methods except AOAC Hard Surface Carrier Test, which is 58/60 carriers are negative for each batch.
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified AOAC Germicidal Spray Products Test or ASTM E2302			
	Towelettes	Modified Germicidal Spray Test			
Broad-spectrum disinfectant	Water soluble powders/liquids	AOAC Use-Dilution Method		Three batches, one at least 60 days old. 60	59/60 carriers are negative for

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Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Evaluation of Success
efficacy products / hard non-porous surfaces	or Internal Toilet or Urinal Bowl Surfaces	or AOAC Hard Surface Carrier Test (distilled water only)	*Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708)	carriers against each organism (360 carriers)	each batch tested for all methods except AOAC Hard Surface Carrier Test, which is 58/60 carriers are negative for each batch.
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified AOAC Germicidal Spray Products Test or ASTM E2362			
Hospital or healthcare disinfectants / hard non-porous surfaces.	Water soluble powders/liquids Or Internal Toilet or Urinal Bowl Surfaces	AOAC Use-Dilution Method or AOAC Hard Surface Carrier Test (distilled water only)	*Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442)	Three batches, one at least 60 days old. 60 carriers against each organism (360 carriers).	59/60 carriers are negative for each batch tested for all methods except AOAC Hard Surface Carrier Test, which is 58/60 carriers are negative against *Staphylococcus aureus* for each batch, and 57/60 carriers are negative against *Pseudomonas aeruginosa*.
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified AOAC Germicidal Spray Products Test or ASTM E2362			
Fungicidal disinfectant/hard non porous surfaces.	Water soluble powders/liquids	AOAC International Fungicidal Activity of Disinfectants TestAOAC Use-Dilution ~~Test modified~~ for fungi or AOAC Fungicidal Test AOAC Use-Dilution Test modified for fungi	*Trichophyton mentagrophytes* (ATCC 9533)	Two batches, three contact times (5, 10 and 15 minutes) per batch. Two batches, ten carriers per batch for the ~~modified~~ AOAC Use-Dilution Test, ~~the modified AOAC Germicidal Spray Products Test, and~~ the EPA disinfectant efficacy test. Note: a reference)) Towelette Test. ~~Two batches for the~~ AOAC Fungicidal Test.	All fungal spores must be killed at 10 and 15 minutes to support a 10 minute claim. All fungal spores on all carriers should be killed.
	Spray products	AOAC Germicidal Spray Products Test modified for fungi		Two batches, ten carriers per batch for the modified AOAC Use-Dilution Test, AOAC Fungicidal Test, the modified AOAC Germicidal Spray Products Test	10/10 carriers are negative for fungal spores for each batch tested.
	Towelettes	Modified AOAC Germicidal Spray Products Test or ASTM E2362AOAC Modified			

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Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Evaluation of Success
		~~Germicidal~~ Spray Test ~~As modified for fungicide towelette testing.~~			
Virucidal disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	~~AOAC Use-Dilution Test modified for viruses or~~ ASTM E1053-~~97~~	Virus claimed on the label or approved surrogate. ~~No specific virus strain is required.~~ ~~Viruses claimed on label must be tested. No seeding claims are required.~~	Two batches. ~~One surface per batch.~~	Complete inactivation of the virus. Where cytotoxicity is present, demonstrate a $3\log_{10}$ reduction ~~beyond the~~ cytotoxic level.
	Spray products	AOAC Germicidal Spray Products Test modified for viruses or ASTM E1053-97			
	Towelettes	ASTM E1053~~AOAC Modified Germicidal~~ Spray Test			
Tuberculocidal disinfectant/hard non-porous surfaces.	Water soluble powders/liquids	AOAC ~~Tuberculocidal~~ Activity of Disinfectants Test	*Mycobacterium bovis* BCG	~~Two batches, ten carriers per batch, 20°C, 10 minute contact time~~	~~10/10 carriers are negative for growth and there is no growth in the additional test media.~~
		AOAC Tuberculocidal Activity of Disinfectants Test with modifications		~~Two batches, ten carriers per batch with modified test conditions~~	~~10/10 carriers are negative for growth and there is no growth in the additional test media.~~
		EPA Quantitative Tuberculocidal Activity Test		~~Two batches, 4 replicates per batch.~~	~~Survival curve constructed from 4 seperate replicates at the 95% confidence level to show probability of one survivor~~
	Spray products	AOAC Germicidal Spray Products Test modified for Tuberculocidal activity		~~Two batches, ten carriers per batch~~	~~10/10 carriers are negative for growth and there is no growth in the additional test media.~~
	Towelettes	~~Modified~~ AOAC Germicidal Spray Products Test or ASTM E1052~~AOAC Modified Germicidal Spray Test~~			

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Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Evaluation of Success
ADDITIONAL ORGANISMS Bactericidal disinfectant/hard non-porous surfaces	Water soluble powders/liquids	AOAC Use-Dilution Test or AOAC Hard Surface Carrier Test (distilled water only)	Organism claimed on the label in addition to the base claim	Two batches, ten carriers for each batch	10/ 10 carriers are negative for growth of the test organism
	Spray products	AOAC Germicidal Spray Products Test			
	Towelettes	Modified AOAC Germicidal Spray Products Test			
Fungicidal disinfectant/hard non-porous surfaces	Liquid Products	AOAC Use-Dilution Test modified for fungi or AOAC Fungicidal Test			
	Spray Products	AOAC Germicidal Spray Products Test modified for fungi			
	Towelettes	Modified AOAC Germicidal Spray Test or ASTM E2362			

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(d) Disinfectants

(1) Limited spectrum products. This section addresses efficacy testing for disinfectant products with limited efficacy (effective against Gram-negative or Gram-positive bacteria, but not both).

(i) Water-soluble powders and ~~non-volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (see reference (i)(1) of this guideline) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Sixty carriers for each of three samples, representing three different batches, one of which must be at least 60 days old~~one of which should be at least 60 days old, should be~~must be tested against *Salmonella enterica* (*S. enterica*) (formerly designated as *Salmonella choleraesuis*)—(ATCC 10708) for effectiveness against Gram-negative bacteria, or *Staphylococcus aureus* (*S. aureus*) (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Sixty carriers for each of three samples, representing three different batches, one of which ~~should~~ must be at least 60 days old, ~~should be~~must be tested against *S. enterica* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *S. aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3)~~,~~ or ASTM E2362 (Ref. 11~~3~~). Sixty carriers for each of three samples, representing three different batches, one of which must be ≥60 days old~~done of which should be at least 60 days old, should be~~must be tested against *S. enterica* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *S. aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass carrier~~slidecarriers, the product ~~should be~~must be tested by wiping the surface of the ~~glass carrier~~slidecarrier with the saturated towelette, and then subculturing the ~~slidecarrier~~carriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should be~~must be used to wipe a minimum of 10 inoculated carriers for a total of 6 towelettes for all 60 carriers~~slidecarriers~~. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers~~slidecarriers~~. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and

documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of limited disinfectant success. For the AOAC International Use-Dilution Methods, the Germicidal Spray Products as Disinfectants test, and single-use towelettes, the product ~~should~~ must kill the test microorganisms on 59 out of each set of 60 /~~slide~~carriers ~~within ten minutes or less~~in ≤10 minutes. ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* must be ≥at least 6.0 (corresponding to a geometric mean density of 1.0×10^6), a mean log density <6.0 invalidates the test.~~ The mean log density for the dried carrier count *S. enterica* must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$); a mean log density <4.0 invalidates the test). For the AOAC International Hard Surface Carrier Test Methods, the product ~~should~~ must kill the test microorganisms on 58 out of each set of 60 carriers for *S. enterica* or *S. aureus* within ten minutes or less. For the alternate one carrier method, the product must completely kill the test organism in ~~within ten minutes or less~~10 minutes.

(2) Confirmatory testing for limited spectrum products

(i) Water-soluble powders and ~~non-volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref.1) or the AOAC International Hard Surface Carrier Test Methods (~~distilled water only~~)(Ref. 2). Ten carriers for each of two product samples, representing two different batches of the product, ~~should be~~ must be tested against either *S. aureus* or *S. enterica* (depending on whether the product is claimed to be effective against Gram-negative or Gram-positive bacteria). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~ must be tested against either *S. aureus* or *S. enterica* (depending on whether the product is claimed to be effective against Gram-negative or Gram-positive bacteria). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. ~~For pressurized spray products, certification should be furnished specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.~~

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~ must be tested against either *S. aureus* or *S. enterica* (depending on whether the product is claimed to be effective against Gram-negative or Gram-positive bacteria). If the product is intended to be represented as bactericidal in the presence of organic

soil (one-step), -an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass carriers~~slidecarrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass~~ slidecarrier with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette must be used to wipe a minimum of 10 inoculated carriers for a total of 6 towelettes for all 60 carriers. ~~One towelette should be used to wipe a minimum of 10 inoculated carriers~~slidecarriers. ~~Alternatively, o~~One carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers~~slidecarriers~~. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of confirmatory limited disinfectant success. The product ~~should~~ must kill all the test microorganisms on all carriers~~slidecarriers~~ within ten minutes or less. ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* must be at least 6.0 (corresponding to a geometric mean density of 1.0×10^6); a mean log density <6.0 invalidates the test.~~ The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$; a mean log density <4.0 invalidates the test).

~~The mean log density for *S. enterica* must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$); a mean log density <4.0 invalidates the test). Need to include discussion on carrier count—need an upper limit. Hard surface carrier test has its own criteria.~~

(3) General or broad spectrum efficacy products. When a disinfectant is represented in labeling as having efficacy against both Gram-negative and Gram-positive bacteria, the product is considered a general or broad spectrum disinfectant.

(i) Water-soluble powders and ~~non-volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Sixty carriers for each of three samples, representing three different batches, one of which ~~should~~ must be at least 60 days old, ~~should~~ must be tested against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Sixty carriers for each of three samples, representing three different batches, one of which ~~should~~ must be at least 60 days old, ~~should~~ must be tested against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5

percent blood serum, ~~should~~ must be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Sixty carriers for each of three samples, representing three different batches, one of which ~~should~~ must be at least 60 days old, ~~should~~ must be tested against both *S. enterica* (ATCC 10708) and *S. aureus* (ATCC 6538). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass carriers~~slide~~carrier~~, the product ~~should~~ must be tested by wiping the surface of the ~~glass carriers~~slidecarrier with the saturated towelette, and then subculturing the ~~carriers~~slidecarriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette must be used to wipe a minimum of 10 inoculated carriers for a total of 6 towelettes for all 60 carriers. ~~One towelette should be used to wipe a minimum of 10 inoculated carriers~~slide~~carriers. Alternatively, a~~One carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriersslidecarriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of general or broad spectrum disinfectant success. For the AOAC International Use-Dilution Methods, the Germicidal Spray Products as Disinfectants test, and single-use towelettes, the product ~~should~~ must kill the test microorganisms on 59 out of each set of 60 carriers~~slide~~carriers within ten minutes or less. ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* must be at least 6.0 (corresponding to a geometric mean density of 1.0×10^6); a mean log density <6.0 invalidates the test.~~ The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of ≥1.0×10^4; a mean log density <4.0 invalidates the test). ~~The mean log density for *S. enterica* must be ≥4.0 (corresponding to a geometric mean density of ≥1.0×10^4); a mean log density <4.0 invalidates the test).~~ For the AOAC International Hard Surface Carrier Test Methods, the product ~~should~~ must kill the test microorganisms on 58 out of each set of 60 carriers within ten minutes or less. ~~Need to comment on carrier count issue – hard surface carrier test has its own range. Need an upper range.~~

(4) Confirmatory testing for general or broad spectrum products

(i) Water-soluble powders and ~~non-volatile~~ liquid products, test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods ~~(distilled water only)~~(Ref. 2). Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~ must be tested against both *S. aureus* and S. *enterica*. If the product is intended to be

represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~ must be tested against both *S. aureus* and S. *enterica*. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. ~~For pressurized spray products, certification should be furnished specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.~~

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~ must be tested against both *S. aureus* and S. *enterica*. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the glass ~~slide~~carrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass carriers~~slide~~carrier~~ with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. The towelette ~~should~~ should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated carriers~~slidecarriers~~. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping ~~should~~ should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers~~slidecarriers~~. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of confirmatory general or broad spectrum disinfectant success. The product ~~should~~ must kill all the test microorganisms on all carriers~~slidecarriers~~ within ten minutes or less. ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* must be at least 6.0 (corresponding to a geometric mean density of 1.0×10^6); a mean log density <6.0 invalidates the test.~~ The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$; a mean log density <4.0 invalidates the test).~~The mean log density for *S. enterica* must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$); a mean log density <4.0 invalidates the test).~~ ~~Need to comment on carrier count issue. hard surface carrier test has its own range. Need an upper range.~~

(5) Hospital or healthcare disinfectants. This section addresses efficacy testing for products recommended for use in hospitals, clinics, dental offices, nursing homes, sickrooms, or any other healthcare-related facility.

(i) Water-soluble powders and ~~non-volatile~~ liquid product test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Sixty carriers for each of three samples, representing three different batches, one of which ~~should~~ must be at least 60 days old, ~~should~~ must be tested against *S. aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (*P. aeruginosa*)(ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Sixty carriers for each of three samples, representing three different batches, one of which ~~should~~ must be at least 60 days old, ~~should~~ must be tested against: *S. aureus* (ATCC 6538), and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Sixty carriers for each of three samples, representing three different batches, one of which must be at least 60 days old, must be tested against *S. aureus* (ATCC 6538), and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass slide~~carrier~~carrier~~, the product ~~should~~must be tested by wiping the surface of the ~~glass carriers~~slide~~c~~carrier with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated carriers~~slide~~carriers. ~~One~~ Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers~~slide~~carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of hospital or healthcare disinfectant success. For the AOAC International Use-Dilution Methods, the Germicidal Spray Products as Disinfectants test, and single-use towelettes, the product ~~should~~ must kill the test microorganisms on 59 out of each set of 60 carriers~~slide~~carriers within ten minutes or less. The mean log density for the dried carrier

count must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4; a mean log density <4.0 invalidates the test). ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* and *P. aeruginosa* must be at least 6.0 (corresponding to a geometric mean density of 1.0 x 10^6); a mean log density <6.0 invalidates the test.~~ For the AOAC International Hard Surface Carrier Test Methods, the product ~~should~~must kill the test microorganisms on 58 out of each set of 60 carriers for *S. aureus*, and 57 out of each set of 60 carriers for *P. aeruginosa* within ten minutes or less.

(6) Confirmatory testing for products with hospital or healthcare disinfectant claim

(i) Water-soluble powders and ~~non-volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods ~~(distilled water only)~~ (Ref. 2). Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~must be tested against *S. aureus* (ATCC 6538) *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~must be tested against *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. ~~For pressurized spray products, certification should be furnished specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.~~

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Ten carriers for each of two product samples, representing two different batches of the product, ~~should~~must be tested against *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass~~ ~~carriers~~slide~~carrier~~, the product ~~should be~~must be tested by wiping the surface of the glass slidecarrier with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should be~~must be used to wipe a minimum of 10 inoculated ~~slidecarriers~~carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of ~~carriers~~slidecarriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and

documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of confirmatory hospital or healthcare disinfectant success. The product ~~should~~ must kill all the test microorganisms on all carriers~~slidecarriers~~ within ten minutes or less. The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4); a mean log density <4.0 invalidates the test). ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* and *P. aeruginosa* must be at least 6.0 (corresponding to a geometric mean density of 1.0 x 10^6); a mean log density <6.0 invalidates the test.~~

(7) Bridging for disinfectant towelettes. In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product forms, the studies in paragraphs (d)(7)(i) and (d)(7)(ii) of this guideline should be conducted and submitted to EPA for review.

(i) Chemical Testing - Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid ~~should be~~must be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package ~~should be~~must be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers can not be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing ~~should~~ must only be that expressed from the towelettes. Three batches (one of which is 60 days old) ~~should~~ must be tested. Analytical data for the active ingredients in the expressed liquid ~~should be~~must be submitted for review.

(ii) Efficacy Testing: Efficacy testing ~~should~~ must be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. ~~Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~ For limited disinfectants, broad-spectrum disinfectants, and hospital disinfectants, to bridge bacterial disinfection claims:

(A) Test Procedure. The Agency recommends the use of the AOAC Germicidal Spray Products as Disinfectants test modified for towelettes, using the test organisms specified for limited, broad-spectrum or hospital disinfectant testing. Sixty carriers for each organism ~~should~~ must be tested against three different batches of the product (one of which ~~should~~ must be at least 60 days old). Instead of spraying the inoculated surface of the ~~glass carriers~~slidecarrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass carriers~~slidecarrier with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. One towelette will be used to treat 10 carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch.

(B) Evaluation of bactericidal towelette success. The product ~~should~~ must kill the test organism on 59 out of 60 carriers. The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$; a mean log density <4.0 invalidates the test). This testing is intended to support bridging of all vegetative bacteria listed on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

(8) Disinfectants for Internal Toilet and Urinal Bowl Surfaces Above and Below the Water Line. This section addresses efficacy testing for products bearing label claims as disinfectants (limited, broad-spectrum, or hospital) for internal toilet and urinal bowl surfaces. Regarding water-soluble powders and ~~non-volatile~~ liquid products test procedure, the Agency recommends the use of the AOAC International Use-Dilution Methods (see reference (i)(1) of this guideline) modified to include a 5% organic soil challenge added to the bacterial inoculum. Sixty carriers for each of three samples, representing three different batches, one of which is must be at least 60 days old, ~~should be~~must be tested against *Salmonella enterica* (ATCC 10708) or *Staphylococcus aureus* (ATCC 6538), for limited disinfectant products; *S. enterica* and *S. aureus*, for broad-spectrum disinfectant products; and *S. aureus* and *Pseudomonas aeruginosa* (ATCC 15442), for hospital disinfectant products. The contained bowl water (~96 fl oz represents traditional high volume toilets) should be used to calculate the appropriate use dilution for testing.

(i) Evaluation of disinfectant success for internal toilet bowl and urinal bowl surfaces. For the AOAC International Use-Dilution Methods ~~and the Germicidal Spray Products as Disinfectants test~~, the product ~~should~~ must kill the test microorganisms on 59 out of each set of 60 carriers~~slidegarriers~~ within ten minutes or less. ~~In addition, per the 2009 revisions for the Use-Dilution Method, the mean log density for *S. aureus* and *P. aeruginosa* must be at least 6.0 (corresponding to a geometric mean density of 1.0×10^6); a mean log density <6.0 invalidates the test.~~ The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$; a mean log density <4.0 invalidates the test). ~~The mean log density for S. enterica must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$); a mean log density <4.0 invalidates the test).~~

(9) Additional microorganisms. This section addresses efficacy testing for limited, broad-spectrum or hospital disinfectants which bear label claims against bacteria other than *Salmonella enterica* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538) or *Pseudomonas aeruginosa* (ATCC 15442).

(i) Water-soluble powders and ~~non-volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Use-Dilution Methods (Ref. 1) or the AOAC International Hard Surface Carrier Test Methods (distilled water only)(Ref. 2). Ten carriers ~~should be~~must be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(ii) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3). Ten carriers ~~should~~ must be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum.

(iii) Single-use towelettes test procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Ten carriers ~~should~~ must be tested against each specific bacterium for each of two samples representing two different batches. If the product is intended to be represented as bactericidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass carrier~~slidecarrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass carrier~~slidecarrier with the saturated towelette, and then subculturing the ~~slidecarriers~~carrier after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated ~~slidecarriers~~carriers. ~~Alternatively, o~~One carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers~~slidecarriers~~. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(iv) Evaluation of disinfectant success for additional microorganisms. The product ~~should~~ must kill all the test microorganisms on all carriers~~/slidecarriers~~ within ten minutes or less. The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4; a mean log density <4.0 invalidates the test).

(c) Disinfectants with fungicidal claims. This section addresses efficacy testing for broad-spectrum or hospital disinfectant products which bear label claims of efficacy against pathogenic fungi.

(1) Water soluble powders and ~~non-volatile~~ liquid products

(i) Test procedures. The Agency recommends the use of the AOAC International Fungicidal Activity of Disinfectants test (Ref. 4). The test is conducted at 5, 10, and 15 minute exposure times. Two samples representing two different batches of the product must be evaluated for efficacy against *Trichophyton mentagrophytes* (*T. mentagrophytes*) (ATCC 9533). The inoculum employed must provide a concentration of > 5 x 10^6 conidia/mL. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum.

The Agency also recommends the use of the AOAC International Use-Dilution Method (Ref. 1). This test may be modified to conform to appropriate elements (e.g., media, growth conditions, etc.) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product must be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed must provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum.

(ii) Evaluation of fungicidal success. For the AOAC International Fungicidal Activity of Disinfectants test, all fungal spores at 10 and 15 minutes must be killed to support a 10 minute exposure time. For the AOAC International Use-Dilution Method, all fungal spores on all 10 carriers must be killed within ten minutes or less.

~~**(c) Disinfectants with fungicidal claims.** This section addresses efficacy testing for broad-spectrum or hospital disinfectant products which bear label claims of efficacy against pathogenic fungi.~~

~~**(1) Water soluble powders and non-volatile liquid products**~~

~~**(i) Test procedures.** The Agency recommends the use of the AOAC International Fungicidal Activity of Disinfectants test (Ref. 4). Two samples representing two different batches of the product should be evaluated for efficacy against *Trichophyton mentagrophytes (T. mentagrophytes)* (ATCC 9533). The Agency also recommends the use of the AOAC International Use-Dilution Methods (Ref. 1). This test may be modified to conform to appropriate elements (e.g., media, growth conditions, etc.) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product should be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum.~~

~~**(ii) Evaluation of fungicidal success.** For the AOAC International Fungicidal Activity of Disinfectants test, all fungal spores in all replicates for each batch should be killed. For the AOAC International Use Dilution Methods, all fungal spores on all 10 carriers should be killed within ten minutes or less.~~

(2) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products—(i) Test procedures. The Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants test (see reference (i)(3) of this guideline). This test may be modified to conform to appropriate elements (e.g., media, growth conditions, etc.) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product ~~should~~ must be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed ~~should be~~must be modified to provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5

percent blood serum, should be included with the fungal inoculum.

(ii) Evaluation of fungicidal success. All fungal spores on all 10 carriers~~/slidecarriers should~~ must be killed within ten minutes or less.

(3) Single-Use Towelettes

(i) Test Procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). ~~The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test.~~ Ten carriers for each of two samples representing two different batches of the product ~~should~~ must be evaluated against *T. mentagrophytes* (ATCC 9533). The inoculum employed ~~should be~~must be modified to provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. If the product is intended to be represented as fungicidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the fungal inoculum. Instead of spraying the inoculated surface of the ~~glass slide~~carrier, the product ~~should be~~must be tested by wiping the surface of the ~~glass slide~~carrier with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated ~~slide~~carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of ~~slide~~carriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(ii) Evaluation of fungicidal towelette success. All fungal spores on all 10 carriers~~/slidecarriers should~~ must be killed within ten minutes or less.

(4) Bridging for disinfectant towelettes. In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (e)(4)(~~i~~i) and (e)(4)(ii) of this guideline should be conducted and submitted to EPA for review:

(i) Chemical Testing - Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid ~~should~~ must be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package ~~should be~~must be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers can not be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing ~~should~~ must only be that expressed from the towelettes. Three batches (one of which is at least 60 days old) ~~should be~~must be tested. Analytical data for the active ingredients in the expressed liquid ~~should be~~must be submitted for review.

(ii) Efficacy Testing: Efficacy testing ~~should be~~must be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. This testing allows bridging of data from the registered bulk liquid used to saturate the towel for each type of organism in this paragraph. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~ For fungicidal test procedure, the Agency recommends the use of the AOAC International Germicidal Spray Products as Disinfectants (Ref. 3) modified for fungicidal towelette testing. The test ~~should be~~must be modified to conform to appropriate elements (e.g. media, growth conditions) in the AOAC International Fungicidal Activity of Disinfectants test. Ten carriers for each of two samples, representing two batches of the product ~~should~~ must be evaluated against *T. mentagrophytes* (ATCC 9533) for the label recommended contact time. The inoculum employed ~~should~~ must be at a count to achieve $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. Instead of spraying the inoculated surface of the ~~glass slide~~carrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass slide~~carrier with the saturated towelette, and then subculturing the ~~slide~~carriers after the specified holding time. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated ~~slide~~carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch.

(A) Evaluation of Fungicidal towelette success. The product ~~should~~ must kill the test organism on all 10 carriers.

(B) Bridging. This testing is intended to support bridging of all fungal test organisms from the EPA registered bulk liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

(f) Disinfectants with virucidal claims. This section addresses efficacy testing for broad-spectrum or hospital disinfectant products that bear label claims of effectiveness against viruses. Virucidal products are intended for use on dry inanimate surfaces; therefore, virological data are usually developed by carrier methods. Each specific virus listed on the label ~~should~~ must be tested. For label claims against Hepatitis B virus, Hepatitis C virus, and Norovirus, the Duck Hepatitis B virus, Bovine Viral Diarrhea virus, and Feline Calicivirus, respectively, are considered acceptable surrogates for testing. Additional guidance and protocols for surrogate virus testing can be found at http://www.epa.gov/oppad001/regpolicy.htm. To simulate in-use conditions, the specific virus to be treated (or surrogate as noted in this paragraph) ~~should be~~must be inoculated onto hard surfaces (e.g., Petri dishes, glass ~~slide~~carriers, ~~stainless steel penicylinders,~~ or other appropriate test surface), allowed to dry, and then treated with the product according to the directions for use on the product label.

(1) Water soluble powders and ~~non-volatile~~ liquid products test procedures. The Agency recommends the use of ~~either the AOAC International Use-Dilution Methods (see reference (i)(1) of this guideline) modified for virucidal testing or~~ the Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces, Designation E1053ASTM E1053-97(current version) Virucidal Test Method (Ref. 5). One surface for each of two samples, representing two different batches of disinfectant, ~~should~~ must be tested against a recoverable

virus end point ~~titer of ≥at least~~ 10^4 viable viral particles from the test surface for a specified exposure period (≤10 minutes) at room temperature. If the product is intended to be represented as virucidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included ~~with~~ with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not ~~expected~~ necessary as part of a study to support a one-step label claim.

(2) Germicidal spray products (aerosol or pump) and ~~volatile~~ liquid products test procedure. The Agency recommends the use of ~~a AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3) modified for virucidal testing or~~ the ASTM E1053-~~97~~ Virucidal Test Method (Ref. 5). One surface for each of two samples, representing two different batches of disinfectant, ~~should~~ must be tested against a recoverable virus end point titer of ~~at least~~ ≥ 10^4 viable viral particles from the test surface for the exposure period specified on the label. ~~If~~ If the product is intended to be represented as virucidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not expected as part of a study to support a one-step label claim.

(3) Single-use towelettes test procedure. The Agency recommends the use of the ASTM E1053 modified for towelettes. One surface for each of two samples, representing two different batches of disinfectant, must be tested against a recoverable virus end point titer of ≥ 10^4 viable viral particles from the test surface for a specified exposure period at room temperature. If the product is intended to be represented as virucidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not expected as part of a study to support a one-step label claim. The towelette should be removed from its container and handled with sterile gloves. One saturated towelette must be used to wipe the surface of each carrier for a minimum of 10 inoculated carriers. One carrier with a surface area equivalent to ten 1 x 1 inch carriers (e.g. ≥ 100 mm petri dish) can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report

~~**(3) Single-use towelettes test procedure.** The Agency recommends the use of the modified AOAC Germicidal Spray Products as Disinfectants test of the ASTM E1053 modified for towelettes. One surface for each of two samples, representing two different batches of disinfectant, should be tested against a recoverable virus end point titer of at least ≥ 10^4 viable viral particles from the test surface for a specified exposure period at room temperature. If the product is intended to be represented as virucidal in the presence of organic soil (one step), an appropriate organic soil, such as 5 percent blood serum, should be included with the viral inoculum. When viral suspensions are grown in the presence of at least 5% serum, addition of serum to the inoculum is not expected as part of a study to support a one-step label claim.~~

~~Instead of spraying the inoculated surface of the glass slidecarrier, the product should be tested by wiping the surface of the glass slidecarrier with the saturated towelette, and then subculturing the slidecarrier, after the specified holding time. The towelette should be removed from its container and subsequently handled with sterile gloves. One towelette should be used to wipe a minimum of 10 inoculated slidecarriers. Alternatively, one carrier with a surface area equivelent to ten 1 x 1 inch carriers (eg., 100 ml petri dish) can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slidecarriers. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process, should be included in the test protocol and documented in the raw data and final report. The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(4) Evaluation of virucidal success. Following treatment of the test virus with the disinfectant product, the presence of remaining viable virus ~~should~~ must then be assayed using an appropriate virological technique (e.g., cytopathogenic effect, fluorescent antibody, plaque count, or animal response). The protocol for the viral assay should provide the information in paragraphs (f)(4)(i)through (f)(4)(ix) of this guideline.

(i) The virus recovery (titer) ~~should~~ must include a minimum of four determinations per each dilution in the assay system (e.g., tissue culture, embryonated egg, animal infection, etc.).

(ii) Cytotoxicity controls. The effect of the disinfectant on the viral assay system ~~should~~ must include a minimum of four determinations per each dilution, however surrogate protocols call for two.

(iii) The activity of the disinfectant against the test virus ~~should~~ must include a minimum of four determinations per dilution in the assay system.

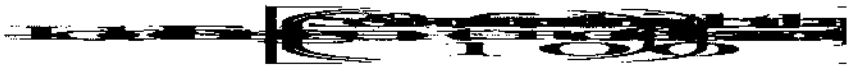
(iv) Neutralization controls. Neutralization controls ~~should be~~must be performed (see reference (i)(6)) and ~~should~~ must include a minimum of four determinations per each dilution however surrogate protocols call for two.

(v) Any special methods which are used to increase the virus titer and to detoxify the residual disinfectant ~~should~~ must be described.

(vi) The ID50 values calculated for each assay ~~should~~ must be provided.

(vii) The test results ~~should~~ must be reported as the reduction of the virus titer by the activity of the disinfectant (ID_{50} of the virus control less the ID_{50} of the test system) expressed as the logarithm to the base 10 and calculated by a statistical method (e.g., Spearman-Karber, Reed and Muench or Most Probable Number).

Spearman-Karber Calculations:



(viii) The product must demonstrate complete inactivation of the virus in all dilutions and ~~with~~ a ~~least a~~ 3-log reduction. If cytotoxicity is present, the virus control titer must be increased to demonstrate ~~at least~~ a 3-$\log_{10}$ reduction beyond the cytotoxic level. Dilutions ~~s~~Showing cytotoxicity or lack of neutralization must not be included in the log reduction calculation. Table 2-1 provides an example of a typical laboratory report of a single test with one virus, assayed in a tissue culture system.

~~(viii) The product should demonstrate complete inactivation with at least a 3-log reduction of the virus at all dilutions. If cytotoxicity is present, the virus control titer must be increased to demonstate at least a 3-$\log_{10}$ reduction in viral titer should be demonstrated beyond the cytotoxic level. Showing cytoticity or lack of neutralization must not be included in the log reduction calculation. Table 1 provides an example of a typical laboratory report of a single test with one virus, assayed in a tissue culture system.~~

(ix) A typical laboratory report of a single test with one virus (recovered from a treated surface) involving a tissue culture assay system would include the details of the methods employed and the information included in Tables 2-1, 2-2 and 2-3:

Table 2-1: Test Results Calculated Using Spearman-Karber Method

Dilution of Virus	Virus - Disinfectant*	Virus - Control*	Cytotoxic – Control
10^{-1}	T T T T	+ + + +	T T T T
10^{-2}	T T T T	+ + + +	T T T T
10^{-3}	T 0 0 0	+ + + +	T 0 0 0
10^{-4}	0 0 0 0	+ + + +	0 0 0 0
10^{-5}	0 0 0 0	+ + + +	0 0 0 0
10^{-6}	0 0 0 0	+ + + 0	0 0 0 0
10^{-7}	0 0 0 0	+ 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
TCID50	≤102.75	106.5	102.75

Note: T = toxic; + = virus recovered; 0 = no virus recovered

Table 2-2: Virus Control Calculation of the Tissue Culture Infective Dose 50 ($TCID_{50}$) utilizing Reed and Muench Calculation (Virus Control Results from Table 2-1)

Values				Accumulated Values			
Virus Dilution Inoculated	No. Infected / No. Inoculated	No. Infected	No. not Infected	No. Infected	No. not Infected	No. Infected / No. Inoculated	% Infected
10^{-1}	4/4	4	0	24	0	24/24	100
10^{-2}	4/4	4	0	20	0	20/20	100
10^{-3}	4/4	4	0	16	0	16/16	100
10^{-4}	4/4	4	0	12	0	12/12	100
10^{-5}	4/4	4	0	8	0	8/8	100
10^{-6}	3/4	3	1	4	1	4/5	80
10^{-7}	1/4	1	3	1	4	1/5	20
10^{-8}	0/4	0	4	0	8	0/8	0

$TCID_{50} = 10^{6.5}$

Table 2-3: Test Calculations of the Tissue Culture Infectivity Dose 50 ($TCID_{50}$) utilizing Reed and Muench Calculation (Test Results from Table 2-1)

Values				Accumulated Values			
Virus Dilution Inoculated	No. Toxic / No. Inoculated	No. Toxic	No. not Toxic	No. Toxic	No. not Toxic	No. Toxic / No. Inoculated	% Toxic
10^{-1}	4/4	4	0	9	0	9/9	100
10^{-2}	4/4	4	0	5	0	5/5	100
10^{-3}	1/4	1	3	1	3	1/4	25
10^{-4}	0/4	0	4	0	7	0/7	0
10^{-5}	0/4	0	4	0	11	0/11	0
10^{-6}	0/4	0	4	0	15	0/15	0
10^{-7}	0/4	0	4	0	19	0/19	0
10^{-8}	0/4	0	4	0	23	0/23	0

$TCLD_{50} = 10^{2.7}$ Therefore: Virus inactivation = $TCID_{50}$ - $TCLD_{50} = 10^{3.8}$ log 10

(5) Bridging for disinfectant towelettes. In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (f)(5)(~~i~~i) and (f)(5)(ii) of this guideline should be conducted and submitted to EPA for review.

(i) Chemical Testing—Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid ~~should~~ must be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package ~~should be~~must be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers can not be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing ~~should~~ must only be that expressed from the towelettes. Two~~hree~~ batches ~~(one of which is at least 60 days old) should~~ must be tested. ~~Analytical data for the active ingredients in the expressed liquid should be~~must be ~~submitted for review~~.

(ii) Efficacy Testing: Efficacy testing ~~should~~ must be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. This testing allows bridging of data from the registered bulk liquid used to saturate the towel for each type of virus in paragraphs (f)(5)(ii)(A)*(1)* through (f)(5)(ii)(A)*(3)* of this guideline. ~~Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(A) Virucidal Test Procedure. The Agency recommends the use of ~~either the AOAC International Germicidal Spray Products as Disinfectants (see reference (i)(3) of this guideline) modified for virucidal towelette testing or~~ Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces, Designation E1053 ~~the ASTM E1053 current edition Virucidal Test Method~~ (see reference (i)(5)) modified for virucidal towelette testing.

(1) To support bridging of all viral claims, the most difficult to inactivate small-sized non-enveloped virus, from the viral strains registered for the bulk liquid, ~~should~~ must be selected for testing. Examples of small-sized non-enveloped viral families include members of the Picornaviridae family (e.g., poliovirus, enterovirus, hepatitis A virus, rhinovirus), and Parvoviridae family (e.g., parvovirus).

(2) To support bridging of viral claims for large-sized non-enveloped and enveloped viral strains, the most difficult to inactivate large-sized non-enveloped virus, from the viral strains registered for the bulk liquid, ~~should~~ must be selected for testing. Examples of large-sized non-enveloped viral families include members of the Adenoviridae family (e.g., adenovirus), Reoviridae family (e.g., rotavirus), and Papillomaviridae family (e.g., papillomavirus).

(3) To support bridging of viral claims for enveloped viral strains, the most difficult to inactivate enveloped virus, from the viral strains registered for the bulk liquid, ~~should~~ must be selected for testing. Examples of enveloped viral families include members of the Coronaviridae family (e.g., coronavirus), Flaviviridae family (e.g., hepatitis C virus), Herpesviridae family (e.g., herpesvirus), Poxviridae family (e.g., vaccinia), Hepadnaviridae family (e.g., hepatitis B virus), Orthomyxoviridae family (e.g., Influenza), Paramyxoviridae family (e.g., parainfluenza) and Retroviridae family (e.g., human immunodeficiency virus).

(B) Ten carriers for each of two samples, representing two batches of disinfectant, ~~should~~ must be tested against a recoverable dried virus titer of ~~≥at least~~ 10^6 viral particles from the test surface for a specified exposure period at room temperature. ~~Instead of spraying the inoculated surface of the glass slidecarrier,~~ The product ~~should~~ must be tested by wiping the surface of the ~~glass slide~~carrier with the saturated towelette~~, and then subculturing the slidecarriers after the specified holding time~~. ~~One towelette should~~ must be used to wipe ~~a minimum of 10 inoculated slides. Alternatively~~, One carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. ~~Alternatively,~~ eOne carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of carriers. A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report.

The protocol for the viral assay should provide the information in paragraphs (f)(5)(ii)(B)*(1)* through (f)(5)(ii)(B)*(7)* of this guideline:

(i) The virus recovery (titer) must include a minimum of four determinations per each dilution in the assay system (e.g., tissue culture, embryonated egg, animal infection, etc.).

(ii) Cytotoxicity controls. The effect of the disinfectant on the viral assay system must include a minimum of ~~two~~four ~~determinations~~ per each dilution however surrogate protocol calls for two.

(iii) The activity of the disinfectant against the test virus must include a minimum of four determinations per dilution in the assay system.

(iv) Neutralization controls. Neutralization controls must be performed (see reference (i)(6)) and must include a minimum of ~~two~~four determinations per each dilution however surrogate protocol calls for two.

(v) Any special methods which are used to increase the virus titer and to detoxify the residual disinfectant must be described.

(vi) The ID50 values calculated for each assay must be provided.

(vii) The test results must be reported as the reduction of the virus titer by the activity of the disinfectant (ID_{50} of the virus control less the ID_{50} of the test system) expressed as the

logarithm to the base 10 and calculated by a statistical method (e.g., Spearmain-Kurber, Reed and Muench or Most Probable Number).

(viii) The product must demonstrate complete inactivation of the virus in all dilutions and a ≥ 3-log reduction. If cytotoxicity is present, the virus control titer must be increased to demonstrate ≥ a 3-$\log_{10}$ reduction beyond the cytotoxic level. Dilutions showing cytotoxicity or lack of neutralization must not be included in the log reduction calculation. Table 2-1 provides an example of a typical laboratory report of a single test with one virus, assayed in a tissue culture system.

~~*(1) The virus recovery (titer) should include a minimum of four determinations for each dilution in the assay system (e.g., cell culture, embryonated egg, animal infection).*~~

~~*(2) Cytotoxicity controls. The effect of the test substance on the viral assay system should include a minimum of four determinations for each dilution.*~~

~~*(3) The activity of the test substance against the test virus should include a minimum of four determinations for each dilution in the assay system.*~~

~~*(4) Neutralization controls. Neutralization controls should be performed (see reference (i)(6)) and should include a minimum of four determinations per each dilution.*~~

~~*(5) Any special methods which are used to increase the virus titer and to detoxify the residual test substance should be described.*~~

~~*(6) The* TCID$_{50}$ or LD$_{50}$ values calculated for each assay should be provided.~~

~~*(7) The test results should be reported as the reduction of the virus titer by the activity of the test substance (LD$_{50}$ of the virus control less the LD$_{50}$ of the test system) expressed as the logarithm to the base 10 and calculated by a statistical method (e.g., Reed and Munch, Most Probable Number or Speer and Carver).*~~

(C) Evaluation of virucidal success. The product must demonstrate complete inactivation of the virus in all dilutions and at ≥ 3-log reduction. If cytotoxicity is present, the virus control titer must be increased to demonstrate ≥ a 3-$\log_{10}$ reduction beyond the cytotoxic level. Dilutions showing cytotoxicity or lack of neutralization must not be included in the log reduction calculation. ~~The product should must demonstrate complete inactivation of the virus at all dilutions. If cytotoxicity is present, at least a 3-log reduction in viral titer should be~~must be ~~demonstrated beyond the cytotoxic level recovered from the carrier surface.~~

(g) Disinfectants with tuberculocidal claims. This section addresses efficacy testing for broad-spectrum or hospital disinfectant products which bear label claims of effectiveness as tuberculocides. In the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims," dated June 13, 1986 (Ref. 8), applicants were given the option of choosing from one of three test methods (AOAC Tuberculocidal Activity of Disinfectants test, a modified AOAC Tuberculocidal Activity of Disinfectants test, or

the Quantitative Tuberculocidal Activity Test) for conducting tuberculocidal efficacy tests. ~~In general, the Agency does not believe that a suspension test is appropriate for disinfectant formulations used on hard surfaces. An exception to this is for glutaraldehyde based products which have never been validated in the AOAC Tuberculocidal Activity of Disinfectants test (a carrier based test). Therefore, the Quantitative Tuberculocidal Activity Test should only be used for glutaraldehyde based products. The Agency strongly recommends all other formulations to use the carrier based AOAC Tuberculocidal Activity of Disinfectants test.~~

(1) Water-soluble powders and ~~non-volatile liquid~~ products test procedures. The Agency recommends the test procedures in paragraphs (g)(1)(i) through (g)(1)(iv) of this guideline.

(i) AOAC International Tuberculocidal Activity of Disinfectants test. The AOAC International Tuberculocidal Activity of Disinfectants test (see reference (i)(8) of this guideline) employing a 10 minute contact time and 20°C temperature. Ten carriers for each of two samples representing two different batches of the product ~~should~~must be tested against *Mycobacterium bovis* (BCG)(*M.bovis*). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, ~~should be~~must be included with the bacterial inoculum. The mean log density for S. cerevisiae *M. bovis* must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4); a mean log density <4.0 invalidates the test). *M. bovis* may not grow in all specified media (AOAC Official Method 965.12 (2008)).

(ii) AOAC International Tuberculocidal Activity of Disinfectants test with modifications. The AOAC International Tuberculocidal Activity of Disinfectants test with modifications to the 10 minute contact time and/or 20°C temperature (see reference (i)(7) of this guideline). Ten carriers for each of two samples representing two different batches of the product ~~should~~ must be tested against *M. bovis* (BCG). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. The mean log density for *M. bovis* must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4); a mean log density <4.0 invalidates the test). *M. bovis* may not grow in all specified media (AOAC Official Method 965.12 (2008)).

(iii) Evaluation of tuberculocide success for (i) and (ii). For the AOAC International Tuberculocidal Activity of Disinfectants test, all organisms on all carriers ~~should~~ must be killed, and there ~~should be~~must be no growth in any of the inoculated subculture media.

(iv) Test Procedure. ~~For glutaraldehyde formulations, t~~The Agency recommends the Quantitative Tuberculocidal Activity Test. This test has been published in the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims", dated June 13, 1986 (Ref. 8). Two samples, representing two different batches of the product must each be utilized in at least four replicate tubes per batch (a total of at least eight replicate studies), against *M. bovis*, so that upper 95 percent confidence limits can be determined for each point on the survival curve. If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5

percent blood serum, must be included with the bacterial inoculum.

(v) Evaluation of tuberculocide success for (iv). For the Quantitative Tuberculocidal Activity Method, survival curves must be constructed from the average of four separate replicates so that the upper 95% Confidence Limit can be determined for each point on the curve. The minimum time claimed for efficacy is determined by finding the point where the average survival curve intersects the probability of one survivor. If the data show a four-log reduction, but the survivor curve does not intersect the one-survivor line, the minimal time is found by extrapolating the upper 95% confidence limit curve such that the value where it intersects the one survivor line is not 50% greater that when the survivor curve intersects the one survivor line.

(ivi) Validation testing for Quaternary Ammonium Compounds. Products formulated solely with quaternary ammonium compounds as the active ingredient(s) ~~should be~~must be supported with validation testing to confirm their tuberculocidal label claim. One additional product sample ~~should~~ must be tested in a different laboratory or in the same laboratory with different study directors and technicians~~staff from the original one~~, using the same test procedure and conditions as used in the ~~first laboratory test~~original test.

~~**(2) Glutaraldehyde formulations**~~

~~**(i) Test Procedure.** For glutaraldehyde formulations, the Agency recommends the Quantitative Tuberculocidal Activity Test. This test has been published in the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness for All Antimicrobial Pesticides with Tuberculocidal Claims", dated June 13, 1986 (Ref. 8). Two samples, representing two different batches of the product should~~must ~~each be utilized in at least four separate studies (a total of at least eight studies), against *M. bovis*, so that upper 95 percent confidence limits can be determined for each point on the survival curve. If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, must~~should be included with the bacterial inoculum.

~~**(ii) Evaluation of tuberculocide success.** For the Quantitative Tuberculocidal Activity Method, survival curves should be~~must be constructed from the average of four separate replicates so that the upper 95% Confidence Limit can be determined for each point on the curve. The minimum time claimed for efficacy is determined by finding the point where the average survival curve intersects the probability of one survivor. If the data show a four-log reduction, but the survivor curve does not intersect the one survivor line, the minimal time is found by extrapolating the upper 95% confidence limit curve such that the value where it intersects the one survivor line is not 50% greater that when the survivor curve intersects the one survivor line.

(3) Germicidal spray products and ~~volatile~~ liquid products—(i) Test procedure. The Agency recommends the AOAC International Germicidal Spray Products as Disinfectants test (Ref. 3), using the media, test culture, and other elements described in the AOAC International Tuberculocidal Activity of Disinfectants test. Ten carriers for each of two samples representing two different batches of the product ~~should~~ must be tested against *M. bovis* (BCG). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step),

an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. The mean log density for *M. bovis* must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4); a mean log density <4.0 invalidates the test). *M. bovis* may not grow in all specified media (AOAC Official Method 965.12 (2008)).

(ii) Evaluation of tuberculocidale success. When using the AOAC International Germicidal Spray Products as Disinfectants test, all organisms on all carriers~~/slidecarriers~~ ~~should~~ must be killed, and there ~~should be~~must be no growth of the test organism in any of the inoculated subculture media. The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4; a mean log density <4.0 invalidates the test).

(4) Single-Use Towelettes

(i) Test Procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11). Ten carriers for each of two samples representing two different batches of the product ~~should~~ must be evaluated against *M. bovis* (BCG). If the product is intended to be represented as tuberculocidal in the presence of organic soil (one-step), an appropriate organic soil, such as 5 percent blood serum, should be included with the bacterial inoculum. Instead of spraying the inoculated surface of the ~~glass~~ slidecarrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass slide~~carrier with the saturated towelette, and then subculturing the slidecarriers after the specified holding time. The towelette should be removed from its container and ~~subsequently~~ handled with sterile gloves. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated ~~slide~~carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of ~~slide~~carriers. ~~Note:~~ A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~ The mean log density for *M. bovis* must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4); a mean log density <4.0 invalidates the test). *M. bovis* may not grow in all specified media (AOAC Official Method 965.12 (2008)).

(ii) Evaluation of tuberculocidal towelette success. All organisms on all carriers~~/slidecarriers~~ ~~should~~ must be killed, and there ~~should be~~must be no growth in any of the inoculated subculture media. The mean log density for the dried carrier count must be ≥4.0 (corresponding to a geometric mean density of ≥1.0 x 10^4; a mean log density <4.0 invalidates the test).

(5) Bridging for disinfectant towelettes. In some cases, disinfectant towelette formulations are identical to registered liquid formulations. In order to bridge efficacy data from the EPA registered bulk liquid disinfectant used to saturate a towelette or other related product form, the studies in paragraphs (g)(5)(i) and (g)(5)(ii) of this guideline should be conducted and submitted to EPA for review.

(i) Chemical Testing - Comparison of Expressed Liquid from the Towelette(s) to the EPA Registered Liquid Disinfectant Formulation to which it is being bridged: All active ingredients in the expressed liquid ~~should be~~must be within the certified limits of the Confidential Statement of Formula of the liquid formula being referenced/bridged. The disinfectant towelettes package ~~should~~ must be filled according to the manufacturing specifications. Excess liquid in the bulk towelette containers can not be poured off for use in the chemical testing for bridging of the efficacy data. The liquid used in the chemical testing ~~should~~ must only be that expressed from the towelettes. ~~Three~~ Two batches ~~(one of which is 60 days old) should be~~must be tested. Analytical data for the active ingredients in the expressed liquid ~~should~~ must be submitted for review.

(ii) Efficacy Testing: Efficacy testing ~~should be~~must be conducted under the same testing conditions (e.g. soil load, contact time, temperature) as used for the bulk liquid testing. This testing allows bridging of data from the registered bulk liquid used to saturate the towel for each type of organism in paragraph (g)(5)(ii)(A) of this guideline. Note: A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. ~~The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.~~

(A) Test Procedure. The Agency recommends the use of a modified AOAC Germicidal Spray Products as Disinfectants test (Ref. 3) or ASTM E2362 (Ref. 11), ~~modified for towelette testing.~~ The test ~~should be~~must be modified to conform to appropriate elements (e.g., media, growth conditions, etc) in the AOAC International Tuberculocidal Activity of Disinfectants test. Ten carriers for each of two samples, representing two batches of the product, ~~should~~ must be tested against *M. bovis* BCG. Instead of spraying the inoculated surface of the glass slidecarrier, the product ~~should~~ must be tested by wiping the surface of the ~~glass slide~~carrier with the saturated towelette, and then subculturing the slidecarriers after the specified holding time. One towelette ~~should~~ must be used to wipe a minimum of 10 inoculated ~~slide~~carriers. Alternatively, one carrier with a surface area equivalent to ten 1 x 1 inch carriers can be wiped using one towelette per carrier set per batch. The mean log density for *M. bovis* must be ≥4.0 (corresponding to a geometric mean density of $\geq 1.0 \times 10^4$); a mean log density <4.0 invalidates the test).

(B) Evaluation of tuberculocidal towelette success. All organisms on all carriers~~/slidecarriers~~ ~~should be~~must be killed, and there ~~should be~~must be no growth in any of the inoculated subculture media.

(C) This testing is intended to support bridging of all mycobacteria listed on the EPA registered liquid disinfectant used to saturate the towelette to the EPA registered towelette product.

(h) Multiple-Use Towelette

The product is intended to be unpackaged and used repeatedly for an extended period until a specified end-point is reached (as determined, for example, by a visible indicator in the product). When a product is intended for a pattern of repeated use, a protocol must be designed which simulates, to the extent possible, the conditions under which it is re-used and to which it

could be exposed, including periodic microbiological challenge, for the duration of its intended use-life. At this point the product is tested to insure its effectiveness in disinfecting hard surfaces.

(1) Simulated Re-Use Protocol. The simulated re-use protocol must include, but is not limited to, the following basic elements:

(i) The cloth must be moistened (in the case of a dry impregnated towelette) and applied to representative type(s) of surfaces as recommended on the label and according to the directions for use. The cloth should then be allowed to partially or completely dry; and the wet-wipe-dry cycle should be repeated until the claimed use-life or specified end-point is reached. These cycles must include periodic challenge with microbiological "bioburden" (viable test bacteria dried onto surfaces/carriers which are wiped). The minimum bioburden load should be equivalent to one glass slide contaminated with > 10^6 viable bacteria (i.e. *Staphylococcus aureu, Salmonella enterica, Pseudomonas aeruginosa*) per each 5 ml of use solution produced in wetting the towelette.

(ii) Periodic chemical monitoring of active ingredient in the use solution produced in the cloth should be performed to show the adequacy and consistency of the concentration provided. In lieu of chemical monitoring, microbiological assay of the surfaces/cloth solution exposed to the bioburden must be performed and found to meet the criteria for acceptable disinfection.

(iii) The use of hard water or organic soil in the re-use protocol would not be required unless label claims are made for use of product in hard water or in the presence of soil ("one-step" cleaning and disinfecting). If such claims are made, the reuse protocol must be conducted with water of the claimed hardness and/or with at least 5% blood serum added to the bacterial inoculum employed as bioburden as well as to the water.

(iv) The specific end-point of the use-life of the towelette is critical, and must be clearly defined on the label for the user and in the protocol for the tester. A comfortable margin of effectiveness must be allowed between the end-point as perceived by the user and the time at which the product is no longer effective as claimed.

(v) At the completion of the simulated re-use protocol, the used towelettes are tested at the specified end-point of their use life for effectiveness as disinfectants as indicated in (1)(a), (1)(b), and (1)(c) above.

~~**(h) Data collection and reporting**~~

~~**(1) General.** To assist in the proper review and evaluation of product performance, complete descriptions of the test employed and the results obtained should be~~must be submitted ~~to the Agency. All test reports should~~ must include, ~~at the least, the information in paragraphs (h)(1)(i) through (h)(i)(xiv) of this guideline:~~

~~**(i) Study title;**~~

~~**(ii) Product identity;**~~

(iii) Guideline number;

(iv) Identification of the testing laboratory or organization;

(v) Location where the test was performed;

(vi) Name(s) of the person(s) responsible for the test;

(vii) 40 CFR Part 160 Good Laboratory Practice compliance;

(viii) Purpose of the study;

(ix) Date and time of the start and end of the test;

(x) Statistical treatment of the data;

(xi) Conclusions;

(xii) References;

(xiii) Appendices;

(xiv) Certification

The applicant is encouraged to use the EPA's standard efficacy report format, which may be found at http://www.epa.gov/oppad001/efficacystudystandards.htm.

(2) Data for recommended methods. When recommended methods from the Official Methods of Analysis of AOAC International; the Annual Book of Standards of the American Society for Testing and Materials; or, EPA methods are used to develop efficacy data, certain minimal information, in addition to that described in this guideline, should must be provided in the test report. The report should mustinclude, at the least, the information in paragraphs (h)(2)(i) through (h)(2)(xii) of this guideline:

(i) Test employed, and any significant modifications thereto (e.g., organic load, hard water);

(ii) Test microorganisms employed, including identification of the specific strain (ATCC or other);

(iii) Description of the test substance, including the percent of active ingredient;

(iv) Concentration or dilution of the product tested and how prepared;

(v) Number of samples, batches and replicates tested;

(vi) Preparation dates of each product batch (individually formulated preparation of the product);

~~(vii) Identification of all material or procedural options employed, where such choice is permitted or recommended in the test method selected (e.g., growth media, drying time for inoculated carriers, neutralization confirmation and/or subculture media, secondary subculturing);~~

~~(viii) Test exposure conditions (e.g., contact time, temperature, and relative humidity);~~

~~(ix) Complete reports of results obtained for each replication;~~

~~(x) Any control data essential to establish the validity of the test;~~

~~(xi) Carrier counts;~~

~~(xii) Any additional data pertinent for specific tests described in this guideline.~~

~~**(3) Data for modifications of recommended methods.** When recommended methods are modified to support specific claims and/or use patterns for a product, the protocol, identifying and describing each modification, should bemust be provided with the study report. The applicant should submit the proposed modification to the Agency for review and evaluation prior to initiation of the test.~~

~~**(4) Data for other methods.** When recommended methods, or modifications thereto, are not employed to develop efficacy data (such as actual in use or many kinds of simulated use testing), complete testing protocols should bemust be submitted with the test reports. All materials and procedures employed in testing should bemust be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should bemust be provided with the test reports. The applicant should mustsubmit the proposed testing protocols for in use or simulated use studies (with a proposed label to show the claims to be supported by the protocol) to the Agency for review and evaluation prior to initiation of the test.~~

(i) References. The references in this paragraph may be consulted for additional background information:

(1) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Use-Dilution Methods (955.14, 955.15, & 964.02), Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(2) *Official Methods of Analysis of the AOAC International,* Chapter 6, Disinfectants, Hard Surface Carrier Test Methods, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(3) *Official Methods of Analysis of the AOAC International,* Chapter 6, Disinfectants, Official Method 961.02 Germicidal Spray Products as Disinfectants, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(4) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 955.17 Fungicidal Activity of Disinfectants. Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(5) *Annual Book of ASTM Standards*, Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces, Designation E1053-~~97~~. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

(6) *Annual Book of ASTM Standards*, Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations, Designation E1483-~~04~~. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

(7) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 965.12 Tuberculocidal Activity of Disinfectants. Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(8) Environmental Protection Agency, Data Call-in Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides with Tuberculocidal Claims (Registration Division, Office of Pesticide Programs, June 13, 1986).

(9) Lennette, E.H., Lennette, D.A. and Lennette, E.T. eds., *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, Seventh Edition, American Public Health Association, Washington, DC.1995.

(10) Spalding Hierarchy Classification

(11) ASTM E2362

CSPA Comments on 810.2300
6/28/2010

OPPTS 810.2300: Sanitizers for use on hard surfaces - efficacy data recommendations.

(a) Scope

(1) Applicability. This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7U.S.C. 136, et seq.), and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 346a). It addresses testing to demonstrate effectiveness of products which ~~are sanitizers~~ have sanitizer claims..

(2) Background. The source materials used in developing this OPPTS test guideline are OPP guidelines 91-2: Products for use on hard surfaces and 91-30: Acceptable methods (Pesticide Assessment Guidelines, Subdivision G, Product Performance. EPA report 540/9-82-026, October 1982).

(b) Purpose. This guideline addresses efficacy testing for antimicrobial pesticides intended to be used on hard surfaces, namely sanitizers in a variety of product types (water-soluble powders, liquids, sprays, towelettes, etc.).

(c) General considerations

(1) This guideline specifies which ~~recommends~~ tests are to be conducted and what data is to be submitted which the Agency believes will generally satisfy the requirements for pesticide registration. All sStudies to be submitted to the Agency for registration which are conducted under this guideline must~~should~~ be completed under EPA's Good Laboratory Practice regulations (40 CFR Part 160). ~~Note: The Association of Official Analytical Chemists (AOAC) recommended tests are expected to be conducted as written.~~ The test methods chosen must be followed to ensure compliance while taking into account application of new technologies, alternate product forms, and specific needs for additional organisms.For deviations ~~(e.g., cultures grown with shaking instead of static, dilution of culture prior to drying on carriers)~~ to the published method proposed to be used in the conduct of these tests, ~~obtain written approval from the Agency and~~ document such deviations in the study reports, submitted to the Agency. ~~The Agency may consult with the AOAC prior to accepting modification to their standardized methods.~~ Refer to OPPTS Test Guideline 810.2000 for general testing recommendations prior to initiating tests.

~~**(2) Confirmatory testing.** In certain situations an applicant may rely on previously submitted efficacy data to support an application or amendment for registration of a product and submit only confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formulation. These situations are as outlined in paragraphs (c)(2)(i) and (c)(2)(ii) in this guideline.~~

~~**(i)** Duplicated Product Formulations. In this situation, the applicant manufactures a~~

~~formulation which duplicates a product that is already registered with complete supporting efficacy data. The chemical composition, manufacturing procedure, label claims, and directions for use are identical in substance to those of the original registration, and specific references (Master Record ID Numbers [MRIDs]) to the supporting data developed for the original product are cited by the applicant.~~

~~(ii) Minor Formulation Change in a Registered Product. In this situation, the change in the formulation is relatively minor, e.g., a change of an inert ingredient. The label claims and directions for use are unchanged from those accepted for the registered formulation, and specific references (MRIDs) to the supporting data developed for the original formulation are cited by the applicant. The confirmatory data must should be developed on the applicant's own finished product. When the test methodology utilized in deriving the original supporting efficacy data were modified to include additional elements not specified in the recommended method, such as organic soil, hard water, longer or shorter contact time, etc., the confirmatory data must should be produced under similarly modified conditions.~~

(~~2~~3) **Efficacy claims.** Table 1 provides a quick reference guide to testing for basic claims described in this guideline. Consult the text for detailed testing descriptions.

Table 1. Testing for ~~basic claims described in this guideline~~ public health sanitizer efficacy claims.

Level of Efficacy	Test Methods		Test Organisms	No. of Batches/Carriers	Eva of Su
Non-food Contact Sanitizer	Water soluble powders/liquids / Spray products	EPA Sanitizer Test, 1976 or ASTM E-1153-~~03~~	*Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048) may be substituted for *K. pneumoniae*.	Three batches, one at least 60 days old. Test 5 carriers per batch per organism, plus 3 non-active control carriers per organism.	≥99.9 redu ~~5 mi~~ each orga para coun minu
	Towelettes	~~Reserved~~ EPA Sanitizer Test, 1976 or ASTM E-1153 method modified for pre-saturated towelettes			
Food Contact Surface Sanitizer Halide Products	Water soluble powders/liquids	AOAC International Chlorine (Available) in Disinfectants Germicidal Equivalent Concentration	*Salmonella typhi (ATCC 6539) ~~or S. aureus (ATCC 6538)~~*	Three batches, one at least 60 days old.	Test must dem prod conc equiv activi 100, ppm chlori
Food Contact Surface Sanitizer Towelettes	Towelettes	http://www.epa.gov/oppad001/towelette.htm	*Escherichia coli (ATCC 11229) and S. aureus (ATCC 6538)*	Three batches, one at least 60 days old.	≥99.9 reduc each micro in ≤ 3
Food Contact Surface Sanitizer Non-Halide Products	Water soluble powders/liquids / Spray products	AOAC International Germicidal and Detergent Sanitizing Action of Disinfectants	*Escherichia coli (ATCC 11229) and S. aureus (ATCC 6538)*	Three batches, one at least 60 days old.	≥99.9 reduc each micro ~~within~~ secor
Sanitizers for Urinal and Toilet Bowl Water and In-tank Sanitizers	Water soluble powders/liquids/tablets	Simulated-use study	*Enterococcus faecalis and or Salmonella enterica*	Three batches, one at least 60 days old.	≥99.9 reduc each organ parali count minut
Residual Self-sanitizing – wet surfaces		Simulated-use study	Representative gram positive and gram negative organisms	Three batches, one at least 60 days old.	≥99.9 reduc each organ parali count minut

(d) Sanitizers for nonfood contact surfaces (water soluble powders, liquids, and

spray products). These products, when used as directed, must ~~should~~ reduce the number of test microorganisms on a treated surface over those of an untreated control surface. The following testing recommendations apply to products bearing label claims for effectiveness as sanitizers for inanimate hard surfaces other than those which come in contact with food or beverages (e.g., floors, walls, furnishings).

(1) Test Procedures.

(i) The Agency recommends the test procedures in this paragraph: The Sanitizer Test for Inanimate Non-food Contact Surfaces (prepared by the Registration Division, Office of Pesticide Programs, EPA, 1976) (Ref. 1). The propagation of cultures and the use of subculture media and other related equipment may be as specified in Official Methods of Analysis of AOAC International, Chapter 6, Disinfectants (Ref. 3). Three product samples, representing three different batches, one of which must ~~should~~ be at least 60 days old, must ~~should~~ be tested against each test bacterium on each representative test surface depending on the uses proposed on the label (for hard, porous surface label claims use unglazed ceramic tile) (for hard, nonporous surface label claims use stainless steel carriers or glass slides), using 5 test carriers and 3 control carriers. The test microorganisms are: *Staphylococcus aureus* (*S. aureus*) (ATCC 6538) and *Klebsiella pneumoniae* (*K. pneumoniae*) (ATCC 4352). *Enterobacter aerogenes* (*E. aerogenes*) (ATCC 13048) may be substituted for *K. pneumoniae*. The test elements in paragraphs (d)(1)(A) through (d)(1)(I) of this paragraph must ~~should~~ be used.

(A) Determine the bacterial count in an 18-24 hour broth culture and add a 0.01- 0.03 mL quantity of the broth culture by spreading on a 1 x 1 inch square or equivalent of test surface using a bacteriological loop.

(B) If the product is intended to be represented as a cleaner-sanitizer, an organic soil load, such as 5 percent blood serum, should be added to the bacterial inoculum.

(C) The square of test surface must ~~should~~ be dried for 35--40 minutes in a bacteriological incubator at 30- 37 °C.

(D) A zero-time bacterial numbers recovery test (dried carrier count) must ~~should~~ be performed to demonstrate the efficiency of the recovery process and must ~~should~~ be reported. The "zero-time" test is intended to show the loss in viability that occurred during carrier drying.

(E) Apply the product to the inoculated test surfaces as directed on the product label.

(F) Run parallel tests on the formulation with the active ingredients omitted in an identical manner to serve as the control. If such a control solution is not suitable, use sterile distilled water to which may be added 0.01 percent isooctylphenoxypolyethoxyethanol (9-10 moles oxyethylene, e.g., Triton X-100).

(G) After the label recommended exposure time, recover the test organisms by washing the squares with agitation in media or dilution fluid containing neutralizers

(H) Make plate counts on nutrient agar containing the same neutralizers by the pour or

spread plate technique.

(I) Exposure time intervals between 0-time and 5 minutes must ~~should~~ be tested for the product as well as the untreated controls.

(ii) The American Society for Testing and Materials (ASTM) Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153-03) may also be used (Ref. 2). ~~Three product samples, representing three different batches, one of which should be at least 60 days old, should be tested against each test bacterium on each representative test surface depending on the uses proposed on the label. (for hard, porous surface label claims use unglazed ceramic tile) (for hard, nonporous surface label claims use stainless steel carrier or glass side), using 5 teset carriers and 3 control carriers. The test microorganisms are: (S. aureus) (ATCC 6538) and K. pneumonia)(ATCC 4352). E aerogenes (ATCC 13048) may be substituted for K. pneumonia.~~ The ASTM method states that the inoculum employed must should provide a count of at least 7.5 x 10^5 colony forming units per carrier.

(iii) Evaluation of sanitizing success for nonfood contact surface sanitizers. The results must ~~should~~ demonstrate a reduction of ~~at least~~ ≥ 99.9% (a 3-log reduction) in the number of each test microorganism over the parallel control count ~~within~~ ≤ 5 minutes.

(e) Towelettes. (Reserved.)

(f) Sanitizers for Internal Toilet and Urinal Bowl Surfaces Above and Below the Water Line

(1) Test Procedures. The Agency recommends the use of the Sanitizer Test for Inanimate Non-food Contact Surfaces (prepared by the Registration Division, Office of Pesticide Programs, EPA, 1976) (Ref. 1), or The American Society for Testing and Materials (ASTM) Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (E1153-03) may be used (Ref. 2). The contained bowl water (96 fl oz)(96. Fl oz represents traditional high volume toilets) must ~~should~~ be used to calculate the appropriate use dilution for testing.

(2) Evaluation of sanitizing success for toilet and urinal bowl surface sanitizers. The results must ~~should~~ demonstrate a reduction of ~~at least~~ ≥99.9% (a 3-log reduction) in the number of each test microorganism over the parallel control count ~~within~~ ≤ 5 minutes.

(g) Sanitizing rinses (soluble powders and liquids) for previously cleaned food-contact surfaces. This section addresses efficacy testing for products with a label recommendation for the treatment of previously cleaned, nonporous, food contact surfaces (e.g., eating and drinking utensils and food processing equipment) as a terminal sanitizing rinse.

(1) Halide chemical products. Sanitizing rinses formulated with iodophors, mixed halides, and chlorine-bearing chemicals.

(i) Test procedure. The Agency recommends the AOAC International Chlorine (Available) in Disinfectants Germicidal Equivalent Concentration test (Ref. 3). Three samples,

representing three different batches, one of which must ~~should~~ be at least 60 days old, must ~~should~~ be evaluated for efficacy against *Salmonella enterica* (*S. enterica*) (formerly Salmonella typhi) (ATCC 6539) or *S. aureus* (ATCC 6538). When claims are made for the effectiveness of the product in hard water, all data must ~~should~~ be developed at the hard water tolerance claimed.

(ii) Evaluation of sanitizing success of halide formulations. Test results must ~~should~~ demonstrate product concentrations equivalent in activity to 50, 100, or ~~and~~ 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

(2) Confirmatory testing for halide chemical products

(i) Test procedure. The Agency recommends the AOAC International Chlorine (Available) in Disinfectants Germicidal Equivalent Concentration test (Ref. 3). One sample must ~~should~~ be evaluated for efficacy against *S. enterica* (ATCC 6539) or *S. aureus* (ATCC 6538). When claims are made for the effectiveness of the product in hard water, all data must ~~should~~ be developed at the hard water tolerance claimed.

(ii) Evaluation of sanitizing success of halide formulations. Test results must ~~should~~ demonstrate product concentrations equivalent in activity to 50, 100, or ~~and~~ 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

(3) Non-halide chemical products. Sanitizing rinses formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations.

(i) Test procedure. The Agency recommends the AOAC International Germicidal and Detergent Sanitizing Action of Disinfectants test (Ref. 4). Three samples, representing three different batches, one of which must ~~should~~ be at least 60 days old, must ~~should~~ be evaluated for efficacy against both *Escherichia coli (E.coli)* (ATCC 11229) and *S. aureus* (ATCC 6538). When claims are made for the effectiveness of the product in hard water, all data must ~~should~~ be developed at the hard water tolerance claimed. If testing with soil load the soil load is added to the inoculum.

(ii) Evaluation of sanitizing success of non-halide formulations. Acceptable results must ~~should~~ demonstrate a $\geq$ 99.999% reduction in the number of each test microorganism within 30 seconds. The results must ~~should~~ be reported according to the actual count and percentage reduction over the control.

(4) Confirmatory testing for non-halide products

(i) Test procedure. The Agency recommends the AOAC International Germicidal and Detergent Sanitizing Action of Disinfectants test (Ref. 4). One sample must ~~should~~ be evaluated for efficacy against both *E. coli* (ATCC 11229) and *S. aureus* (ATCC 6538). When claims are made for the effectiveness of the product in hard water, all data must ~~should~~ be developed at the hard water tolerance claimed. If testing with soil load the soil load is added to the inoculum.

(ii) Evaluation of sanitizing success of non-halide formulations. Acceptable results must ~~should~~ demonstrate a ≥99.999% mean reduction in the number of each test microorganism within 30 seconds. The results must ~~should~~ be reported according to the actual count and percentage reduction over the control.

(h) Towelette Sanitizers for Food Contact Surfaces. This section addresses efficacy testing for products with a label recommendation for the treatment of hard, non-porous surfaces which may come into contact with food. Food Contact Surface (FCS) towelettes are intended to be used to sanitize the following surfaces: hard non-porous tables, countertops (stainless steel, laminated, sealed ceramic,) stove tops, interior and exterior surfaces of microwaves and refrigerators and other appliances. FCS towelettes may not be used to sanitize the following food contact surfaces: utensils, glasses, food containers, dishes, unfinished wood cutting boards, unfinished wood cutting blocks, drain boards, and food processing equipment. This list is not meant to be all-inclusive, but to serve as general guidance for the appropriate use of this type of antimicrobial pesticide. The Agency reserves the right to accept or deny use sites for food contact surface towelettes on a case-by-case basis.

(1) Test Procedure. The Agency recommends the use of the Interim Guidance for Non-Residual Sanitization of Hard Inanimate Food Contact Surfaces Using Pre-Saturated Towelettes. This guidance may be found at: http://www.epa.gov/oppad001/towelette.htm. If testing with soil load the soil load is added to the inoculum.

Three samples, representing three different batches, one of which ~~is~~ must be at least 60 days old, must ~~should~~ be evaluated for efficacy against *E. coli* (ATCC 11229) and *S. aureus* (ATCC 6538).

(2) Evaluation of towelette sanitizing success. The product must ~~should~~ demonstrate ~~at least~~ a ≥99.999% mean reduction in the number of test microorganisms (bacteria) within 30 seconds. The result must ~~should~~ be reported according to the actual count and percentage reduction over the control.

(i) Sanitizers for Toilet and Urinal Bowl Water. This section addresses efficacy testing for products with claims as sanitizers for toilet and urinal bowl water.

(1) Test Procedure. A simulated-use study must ~~should~~ be designed which incorporates all of the elements listed in paragraphs (i)(1)(i) through (i)(1)(iv) in this guideline.

(i) The product must ~~should~~ be added to samples of the bowl water from three toilets or urinals, one toilet per lot, at the use concentration employing the recommended method of dispensing. Untreated control samples from the three toilets or urinals must ~~should~~ also be included.

(ii) Whether the product is automatically metered, or dispensed in some other fashion, into the bowl water (or urinal trap), ~~the consistent accuracy of~~ the concentration of the bowl

water must be ~~dispensed and~~ maintained ~~should be~~and documented.

(iii) Inocula containing representative Gram-positive or Gram-negative test bacteria must ~~should~~ be added to the treated and control samples of the bowl water from each of the toilets or urinals to provide a concentration of at least $\geq 10^4$ colony-forming units per milliliter. If testing with soil load the soil load is added to the inoculum.

(iv) Microbial counts of the treated bowl water and the control bowl water must ~~should~~ be conducted at a minimum of three exposure intervals, in addition to a 0-time control.

(2) Evaluation of Sanitizing Success. The reduction of each test microorganism must ~~should~~ be at ~~least~~ ≥99.9% mean reduction over the 0-time control and the parallel untreated inoculated control.

(j) In-Tank Sanitizers. This section addresses efficacy testing for products which bear label claims for use as an automatically dispensed in-tank sanitizer.

(1) Test Procedures. In-tank sanitizer products must ~~should~~ be evaluated by a preliminary simulated-use test followed by a laboratory efficacy test. These tests must ~~should~~ incorporate the elements in paragraphs (j)(1)(i) through (j)(1)(i)(B).

(i) Preliminary simulated-use test. The use-life of the in-tank product must ~~should~~ be documented for three product samples, each in a separate toilet under the conditions in paragraphs (j)(1)(i)(A) and (j)(1)(i)(B) of this guideline simulating actual usage. Testing conducted with a 6 gallons/flush toilet may be used to generate data for low flush toilets (3.5 gallons/flush).

(A) Number of flushes (dispensation of the dosage) per day per X weeks (duration of effectiveness) with a non-chlorinated water supply at 25-30 °C (the warm water temperature extreme in summer).

(B) The bowl water must ~~should~~ be analyzed at periodic intervals during the testing indicated in paragraph (j)(1)(i)(A) in this guideline to demonstrate the pH and concentration of the active ingredients.

(ii) Laboratory efficacy tests. Bacteriologic assays must be conducted on ~~neutralized~~ treated and untreated samples which are neutralized and plated by standard ~~plating~~ procedures employing:

(A) Samples of the residual bowl water from three toilets (one lot per toilet) (at the minimal use concentration) and corresponding untreated control samples from three toilets at 10-.15 °C (the most stringent water temperature for demonstrating efficacy).

(B) Representative Gram-positive and Gram-negative bacteria (e.g., *Enterococcus faecalis, Salmonella enterica)* with an inoculum of ~~at least~~ $\geq 10^4$CFU/mL.must be used.

(C) A minimum of three exposure intervals, in addition to a 0-time control.

(iii) Evaluation of in-tank sanitizing success. The reduction of each test microorganism must ~~should~~ be ~~at least~~ ≥99.9% mean reduction over the 0-time control and the parallel untreated inoculated control.

(k) Residual self-sanitizing activity of dried chemical residues on hard-inanimate surfaces - wet surfaces. This section addresses efficacy testing for products which bear label claims to provide residual self-sanitizing activity (e.g., significant reduction in numbers of infectious microorganisms which may be present or subsequently deposited) on treated surfaces that are likely to become and remain wet under normal conditions of use.

(1) Test procedure. Residual self-sanitizing products for use on hard, non-porous ~~inanimate~~ surfaces must ~~should~~ be evaluated for efficacy using a controlled in-use study or simulated in-use study. The design of the study must ~~should~~ be done in consultation with the Agency and must ~~should~~ include the basic elements: in paragraphs (k)(1)(i) through (k)(1)(vii) of this guideline.

(i) The test microorganisms employed in the study must ~~should~~ be pathogens that are likely to be encountered in the environment in which the product is to be used.

(ii) The starting inocula of the test microorganisms for both initial and subsequent challenges must ~~should~~ be of sufficient concentration to provide ~~at least~~ $\geq 10^4$ survivors on the parallel control surface.

~~(iii) Subsequent challenges should be of sufficient frequency to accurately represent normal conditions of use.~~

~~(iv~~iii) Quantitative bacteriological sampling must ~~should~~ be conducted at frequent and regular intervals for the length of time the residual activity can be expected to exist under the expected use conditions.

(~~v~~iv) The same types of surfaces without the treatment must ~~should~~ be employed in the test and inoculated in a manner and over an exposure period identical to the use pattern for which the product is intended.

(~~vi~~v) The environmental conditions (e.g., relative humidity and temperature) must ~~should~~ be the same as those likely to be encountered under normal conditions of product use. Tests must ~~should~~ also include those environmental conditions that would act to reduce the effective concentration of the product on the inanimate surface (e.g., rinsing, abrasion, organic load, repeated challenges by microorganisms, etc.).

(vii) The length of time the residual activity can be expected to exist under the expected use conditions must ~~should~~ be documented.

(2) Evaluation of success of residual self-sanitizing action. For residual self-sanitizing claims, it must ~~should~~ be demonstrated that a product is capable of reducing the number of test microorganisms on the test surface by ≥99.9% over that of the parallel control surfaces.

(l) Residual self-sanitizing activity of dried chemical residues on hard-inanimate surfaces - dry surfaces. This section addresses efficacy testing for products which bear label claims to provide residual self-sanitizing activity (e.g., significant reduction in numbers of infectious microorganisms which may be present or subsequently deposited) on treated dry surfaces.

(1) Test Procedure. The Agency recommends the use of the Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard Non-Porous Surfaces. ~~This guidance~~ Agency accepted protocols may be found at http://www.epa.gov/oppad001/regpolicy.htm

(2) Evaluation of residual self-sanitizing success. The product must ~~should~~ demonstrate that it is capable of reducing the number of test microorganisms on the test surface by ≥99.9% over that of the parallel control surfaces ~~within~~ > 5 minutes for a specified time period as demonstrated by the method, eg 12 hours, 24 hours, etc. ~~period.~~.

~~**(m) Data collection and reporting**~~

~~**(1) General.** 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, what is in paragraphs (m)(1)(i) through (m)(1)(xiv) of this guideline:~~

~~**(i)** Study title;~~

~~**(ii)** Product identity;~~

~~**(iii)** Guideline number;~~

~~**(iv)** Identification of the testing laboratory or organization;~~

~~**(v)** Location where the test was performed;~~

~~**(vi)** Name(s) of the person(s) responsible for the test;~~

~~**(vii)** Good Laboratory Practice compliance;~~

~~**(viii)** Purpose of the study;~~

~~**(ix)** Date of the start and end of the test;~~

~~**(x)** Statistical treatment of the data;~~

~~(xi) Conclusions;~~

~~(xii) References;~~

~~(xiii) Appendices~~

~~(xiv) Certification~~

~~The applicant is encouraged to use the EPA's standard efficacy report format, which may be found at http://www.epa.gov/oppad001/efficacystudystandards.htm~~

(~~n~~m) **References:** The following references may be consulted for additional background information:

(1) Environmental Protection Agency, *Sanitizer Test for Hard, Inanimate Nonfood Contact Surfaces Modified to Include Organic Soil.* (Registration Division, Office of Pesticide Programs, 1976).

(2) *Annual Book of Standards*, Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, Designation E1153-03. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

(3) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 955.16 Chlorine (Available) in Disinfectants, Germicidal Equivalent Concentration. Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(4) *Official Methods of Analysis of the AOAC International*, Official Method 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants. Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(5) General Requirements for Antimicrobial Agents (Pesticide Assessment Guidelines, Subdivision G, Product Performance, EPA report 540/9-82-026, October 1982).

(6) Antimicrobial Science Policies; Disinfectant Technical Science Section (DIS/TSS); http://www.epa.gov/oppad001/sciencepolicy.htm.

DRAFT

OPPTS 810.XXXX Air Sanitizers

Refer to OPPTS 810.2000 for general testing recommendations prior to initiating tests.

(a) Scope

(1) Applicability – This guideline describes test protocols that EPA believes will generally satisfy product performance test requirements of the Federal Insecticide, Fungicide, and Rodenticide ACT (FIFRA) (7 U.S.C. 136, et seq.). It addresses public health and non-public health air sanitizers.

(2) Background – The source materials used in developing this OPPTS test guideline are Pesticide Assessment Guidelines (Subdivision G, Product Performance, EPA Report 540/9-82-026, October 1982), EPA DSS/TSS and applicable PR Notices.

(b) Introduction – This document addresses efficacy testing for air sanitizers. These requirements apply to products with label claims for the treatment of air to reduce the numbers of airborne microorganisms. This guideline recommends tests to be conducted and data to be submitted which the Agency believes will generally satisfy the requirements for pesticide registration. The Agency recognizes that novel technologies associated with antimicrobial products may evolve over time and may require test methods that are not included in this current guideline. The Agency intends to update these guidelines periodically. However, new methods must be approved prior to guideline updates.

Air Sanitizers

(i) Products containing glycols

(A) General – There is considerable evidence[1] that glycol vapors produce significant decreases in the number of viable airborne bacteria under relatively wide conditions of relative humidity and temperature when properly and continuously

[1] See the references given at the end of this document.

dispensed by a vaporizing device that maintains suitable concentrations in the air of enclosed spaces. With dispensers for the intermittent treatment of air, such as pressurized aerosols, several investigators have shown that glycols (triethylene, dipropylene, or propylene glycol) at concentrations of 5% or more in such formulations will temporarily reduce numbers of airborne bacteria when adequate amounts are dispensed under relatively ideal conditions.

(B) Test Procedure – No standard analytical method for evaluating glycol air saturation has been adopted. The following criteria apply for products containing glycols:

(1) The product must contain at least 5% glycols (triethylene, dipropylene and/or propylene glycols). This requirement is satisfied by the confidential statement of formula showing the appropriate glycol content.

(2) Claims must clearly indicate the mitigating nature of the activity, such as "Temporarily reduces the number of airborne bacteria".

(3) The Directions for Use of air sanitizers must state:

- That application must be made in closed spaces, for example, 'Close all doors and windows, close air vents or turn off air conditioners'.

- The duration and frequency of spraying.

- The volume of space to be treated.

- How the spraying should be conducted.

- The relative humidity necessary for maximal effectiveness.

(4) With glycols and glycol mixtures intended for use in continuous vaporizing devices, the vaporizers themselves do not require registration. However, since

their use is an integral part of the directions for use of the pesticide product, the labeling used in connection with the sale of the vaporizer is collateral to the labeling of the pesticide and must be submitted as part of the registration.

(C) Evaluation of Glycol Air Sanitizer Success – A 50% glycol saturation or more must be achieved when the product is used in accordance with its label.

(ii) Products not containing glycols

(A) General – For products intended for the treatment of air which do not contain glycols, claims for reducing the number of airborne microorganisms will be considered, provided that supporting experimental data are submitted to justify such claims.

(B) Test Procedure – No standard microbiological efficacy method for evaluating air sanitizers has been adopted. Refer to the attached references for information on testing products intended for sanitizing the air of enclosed spaces. Proposed testing protocols for studies of this kind may be submitted for review and evaluation by the Agency prior to the initiation of the tests (see EPA website for document entitled 'Internal Antimicrobials Division (AD) Guidance on Review of Efficacy Testing Protocols Intended to Support the Registration of Antimicrobial Products, July 13 2001'). The following criteria apply for products which do not contain glycol:

(1) Quantitative microbiological assays must be performed, using an air sampling device, to show the level of reduction of viable microorganisms achieved with the product, when used as directed, in an enclosed experimental room or chamber. The primary test bacteria are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 44352). If the product is intended for use in hospitals or medical environments, testing with *Pseudomonas aeruginosa* (ATCC 15442) are also required.

(2) The methodology employed, such as spraying and sampling procedures, and the environmental conditions in the room or chamber, such as

temperature and relative humidity, must be reported. The settle rates in the air of the test enclosure must be measured for each of the required test bacteria. The study design must include a parallel, untreated control. Raw data, as well as any statistical or graphical interpretation of the results, must be included in the
reports.

(3) Thus, claims of value in preventing or treating diseases, or providing any other health protection, whether expressed or implied, are not acceptable. Claims must clearly indicate the mitigating nature of the activity, such as "Temporarily reduces the number of airborne bacteria".

(4) The label directions for use of air sanitizers must state:

- That application must be made in closed spaces, for example, 'Close all doors and windows, close air vents or turn off air conditioners'.

- The duration and frequency of spraying.

- The volume of space to be treated.

- How the spraying should be conducted.

- The relative humidity necessary for maximum effectiveness.

(C) Evaluation of Non-glycol Air Sanitizer Success – The results must show a viable count reduction of at least 99.9% over the parallel untreated control, after correcting for settling rates in the air of the test enclosure with each of the required test bacteria.

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DRAFT

OPPTS 810.XXXX Antimicrobials for use on Fabrics, Textiles and Carpeting

Refer to OPPTS 810.2000 for general testing recommendations prior to initiating tests.

(a) Scope

(1) Applicability – This guideline describes test protocols that EPA believes will generally satisfy product performance test requirements of the Federal Insecticide, Fungicide, and Rodenticide ACT (FIFRA) {7 U.S.C. 136, et seq.}. It addresses antimicrobials used on fabrics, textiles and carpeting.

(2) Background – The source materials used in developing this OPPTS test guideline are Pesticide Assessment Guidelines (Subdivision G, Product Performance, EPA Report 540/9-82-026, October 1982), EPA DSS/TSS and applicable PR Notices.

(b) Introduction – This guideline addresses efficacy testing for antimicrobial products to be used on fabrics, textiles, and carpeting. This guideline recommends tests to be conducted and data to be submitted which the Agency believes will generally satisfy the requirements for pesticide registration. The Agency recognizes the fact that novel technologies associated with antimicrobial products may evolve over time and may produce test methods that are not included in this current guideline. The Agency intends to update these guidelines periodically. The use of new methods may be approved prior to guideline updates.

Laundry Additives

(i) Laundry Operations Considerations – A clear distinction should be made on the label between products recommended for household and coin-operated laundering and products represented as commercial-industrial-institutional laundry additives. The effectiveness of an antimicrobial laundry product may be altered by differences in laundry machine types (top loading vs. high efficiency machines). Therefore, the type of machine should be taken into consideration. The water to fabric ratio in common top loading laundry

machines is approximately 10:1 (wash volume to fabric weight), while with the new high efficiency laundry machines, it can be as low as approximately 2.5:1. It must be noted that machine manufacturers are constantly upgrading machines and therefore the fabric to water ratios noted above are to be considered as examples. Directions for use of household laundering products may require different dosages for front-loading machines (e.g. 8-10 gallon water capacity) and top-loading machines (e.g. 12-15 gallon water capacity.) Household product dosages should be specified in household measurements. Industrial product dosages may be based on pounds of dry fabric.

(ii) Sterilant Pre-soak Treatments

(A) Test Procedure -The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants Method, in the presence of at least 5% organic soil (e.g. blood serum) modified for use on fabric carriers). The following elements must be incorporated into each study:

(1) The directions for use must specify rinsing of the items to remove gross filth prior to soaking, followed by complete immersion in an adequate volume of soaking solution (at least 5:1 w/w solution to fabric ratio, e.g. half a wash load in a 3 gallon pail) at the recommended use dilution at a specific water temperature for a specified contact time prior to the laundering application.

(2)Sixty carriers should be tested against spores of both Bacillus subtilis (American Type Culture Collection (ATCC) 19659) (B. subtilis) and Clostridium sporogenes (ATCC 3584) (C. sporogenes) on three samples representing three different batches of the product, one of which should be at least 60 days old (240 carriers per sample, or a total of 720 carriers). Modifications to the AOAC Sporicidal Activity of Disinfectants test to address this use should be submitted to the Agency for review and approval prior to conducting the tests. The inoculum employed should provide a count of 1×10^4 - 5×10^6 spores per carrier.

B. Confirmatory testing for Clostridium difficile -The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants Method, in the presence of at least 5% organic soil (e.g. blood serum) modified for use with fabric carriers. The following elements must be incorporated into each study:

(1) Thirty carriers should be tested against spores of Clostridium difficile on two samples representing two different batches of the product. Modifications to the AOAC Sporicidal Activity of Disinfectants test to address this use should be submitted to the Agency for review and approval prior to conducting the tests. The inoculum employed should provide a count of 1×10^4 - 5×10^6 spores per carrier.

C.) Evaluation of Sterilant Pre-soak Treatment Success – The product must kill the test microorganisms on all carriers of each set.

(iii) Disinfecting Pre-soak Treatments

(A) Test Procedure – Disinfectant products recommended for pre-soaking soiled fabrics prior to routine laundering must be shown to be effective using the AOAC Use Dilution Method in the presence of at least 5% organic soil (e.g. blood serum) modified for use on cotton carriers. The following elements must be incorporated into each study:

(1) The directions for use must specify rinsing of the items to remove gross filth prior to soaking, followed by complete immersion in an adequate volume of soaking solution (at least 5:1 w/w solution to fabric ratio, e.g. half a washload in a 3 gallon pail) at the recommended use dilution at a specific water temperature for a specified contact time prior to the laundering application.

(2)). Nine swatches (carriers) should be tested against each bacteria. The test bacteria are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352)or Enterobacter aerogenes (ATCC 13048). If the product is intended for use on hospital linens, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442).

(3) The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier. Testing should be conducted on three samples representing three different batches of the product, one of which should be at least 60 days old.

(B) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the AOAC Use Dilution Method in the presence of at least 5% organic soil (e.g. blood serum) (see paragraph (A) of this guideline). Nine swatches (carriers) should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(C) Additional Organisms – Fungal – The Agency recommends the use of the AOAC Use Dilution Method in the presence of at least 5% organic soil (e.g. blood serum) (see paragraph (A) of this guideline). Nine swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(D) Additional Organisms – Viral - The Agency recommends the use of the AOAC Use Dilution Method, modified for viruses on fabric, in the presence of at least 5% organic soil (e.g. blood serum) (see paragraph (A) of this guideline). Nine swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of a minimum of 1×10^4 ID_{50} per carrier.

(E) Evaluation of Disinfecting Pre-soak Treatment Success – The product must kill the test microorganisms on all nine out of each set of nine carriers.

(iv) Sanitizing Pre-soak Treatments

(A) Test Procedure - Sanitizing products recommended for pre-soaking soiled fabrics prior to routine laundering must be shown to be effective using the Sanitizer Test for inanimate non-food contact surfaces in the presence of at least 5% organic soil (e.g. Horse serum) (ASTM E1153-03) modified for use on cotton carriers. Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-

Food Contact Surfaces). The following elements must be incorporated into each
study:

(1)The directions for use must specify rinsing of the items to remove gross filth prior to soaking, followed by complete immersion in an adequate volume of soaking solution (at least 5:1 w/w solution to fabric ratio, e.g., half a washload in a 3 gallon pail) at the recommended use dilution at a specific water temperature for a specified contact time prior to laundering.

(2) Three swatches (carriers) should be tested against each bacteria. The test bacteria are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352) or Enterobacter aerogenes (ATCC 13048) If the product is intended for use on hospital linens, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442).

(3) Parallel tests on the formulation with the active ingredients omitted in an identical manner serve as the control. If such a control solution is not suitable, sterile distilled water or sterile distilled water to which 0.01percent isooctylphenoxypolyethoxyethanol (9-10 moles oxyethylene, e.g., Triton X-100, Tween) may be added.

(4) Testing should be conducted on three samples representing three different batches of the product, one of which should be at least 60 days old. Calculate the log reduction for each test lot from the geometric mean of the survivors from the three test carriers as compared with the three untreated parallel control carriers.

(B) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the Sanitizer Test for inanimate non-food contact surfaces in the presence of at least 5% organic soil (e.g. Horse serum) (ASTM E1153-03 Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces)(see paragraph (A) of this guideline). Three swatches (carriers) should be tested against each specific bacterium for each of two samples representing two different batches. The

inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(C) Additional Organisms – Fungal – The Agency recommends the use of the Sanitizer Test for inanimate non-food contact surfaces in the presence of at least 5% organic soil (e.g. Horse serum) (ASTM E1153-03 Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces) (see paragraph (A) of this guideline). Three swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(D) Additional Organisms – Viral - The Agency recommends the use of the Sanitizer Test for inanimate non-food contact surfaces in the presence of at least 5% organic soil (e.g. Horse serum) (ASTM E1153-03 Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces) (see paragraph (A) of this guideline). Three swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of a minimum of 1×10^4 ID_{50} per carrier.

(E) Evaluation of Sanitizing Pre-soak Treatment Success – At least a 3 log reduction compared to the parallel control count must be demonstrated against each test organism.

(v) Disinfecting Laundry Additives (non-residual)

(A) Test Procedure – ASTM Methods E 2274 and E 2406 are acceptable. Alternatively, a simulated-use study utilizing washing machines may be employed. The following elements must be incorporated into each study:

(1) The test bacteria are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). If the product is intended for use on hospital linens, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442)

(2) Tests must be conducted with 3 product samples, representing 3 different batches, one of which is at least 60 days old. Each sample must be

tested with 9 fabric swatches against each of the test bacteria.

(3) The method employed must be designed to include testing both the fabric and the laundry water (5mL from the automatic washer, or 0.5 mL from the simulated washing device in individual widemouth jars containing subculture media and neutralizers). The laundry water-to-media volume ratio must not exceed 1:40. The effectiveness of an antimicrobial laundry product may be altered by differences in laundry machine types (top loading vs. high efficiency machines). Therefore, the type of machine should be taken into consideration. The water to fabric ratio in common top loading laundry machines is approximately 10:1 (wash volume to fabric weight), while with the new high efficiency laundry machines, it can be as low as approximately 2.5:1. It must be noted that machine manufacturers are constantly upgrading machines and therefore the fabric to water ratios noted above are to be considered as examples.

(4) Growth or no-growth must be recorded and reported after a 48-hour incubation period.

(5) The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(B) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of ASTM Methods E 2274 and E 2406 (see paragraph (A) of this guideline). Nine swatches (carriers) should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(C) Additional Organisms – Fungal - The Agency recommends the use of ASTM Methods E 2274 and E 2406 (see paragraph (A) of this guideline). Nine swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(D) Additional Organisms – Viral - The Agency

recommends the use of ASTM Methods E 2274 and E 2406 (see paragraph (A) of this guideline). Nine swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of a minimum of 1×10^4 I_d 50 per carrier.

(E) Evaluation of Disinfecting Laundry Additives Success (non-residual)– There must be no growth in the fabric subcultures and no growth in the subcultures from the laundry water with all test bacteria and viruses.

(F) Special Note – The data requirements outlined herein do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones or trichloro-s-triazinetrione.

Comment [E1]: We request additional discussion and explanation of this historical note. We are not aware of supporting rationale for this text. We suggest that the note provide further explanation of why the methods are not applicable, cite alternative test methods for these active ingredients, or remove the note.

(vi) Sanitizing Laundry Additives (non-residual)

(A) Test Procedure – ASTM Methods E 2274 and E 2406 are acceptable. Alternatively, a simulated-use study utilizing washing machines may be employed. The following basic elements must be incorporated in each study:

(1) The test bacteria are *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352. If the product is intended for use on hospital linens, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442)

(2) The basic bacteriological procedures must be the same as those specified in the ASTM Methods E 2274 and E 2406.

(3) Tests must be conducted with 3 samples representing 3 product batches, one of which is at least 60 days old. Each sample must be tested with 5 cotton swatches against each test microorganism required.

(4) The method employed must be designed to include testing both the fabric and the laundry water (5ml from the automatic washer, or 0.5 ml from the simulated washing device in individual widemouth jars containing subculture media and neutralizers). The laundry water-to-media volume ratio must not exceed

1:40. The effectiveness of an antimicrobial laundry product may be altered by differences in laundry machine types (top loading vs. high efficiency machines). Therefore, the type of machine should be taken into consideration. The water to fabric ratio in common top loading laundry machines is approximately 10:1 (wash volume to fabric weight), while with the new high efficiency laundry machines, it can be as low as approximately 2.5:1. It must be noted that machine manufacturers are constantly upgrading machines and therefore the fabric to water ratios noted above are to be considered as examples.

(5) Quantitative bacteriological assays must be conducted and the results reported.

(6) Calculate the log reduction for each test lot from the geometric mean of the survivors from the test as compared with the untreated parallel control carriers.

(B) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of ASTM Methods E 2274 and E 2406 (see paragraph (A) of this guideline). Three swatches (carriers) should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(C) Additional Organisms – Fungal – The Agency recommends the use of ASTM Methods E 2274 and E 2406 (see paragraph (A) of this guideline). Three swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of $1 \times 10^4 - 5 \times 10^6$ colony forming units per carrier.

(D) Additional Organisms – Viral - The Agency recommends the use of ASTM Methods E 2274 and E 2406 (see paragraph (A) of this guideline). Three swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of a minimum of 1×10^4 ID_{50} per carrier.

(E) Evaluation of Sanitizing Laundry Additives (non-residual) – At least a 3 log reduction in test organism compared to the control count for both laundry water and fabric must be demonstrated against each test organism.

(F) Special Note – The data requirements outlined herein do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones or trichloro-s-triazinetrione.

Comment [E2]: We request additional discussion and explanation of this historical note. We are not aware of supporting rationale for this text. We suggest that the note provide further explanation of why the methods are not applicable, cite alternative test methods for these active ingredients, or remove the note.

(vii) Self-sanitizing Laundry Additives (residual) and Microbiostasis (residual)

(A) General - The following requirements apply to products which bear claims or recommendations for use in the treatment of laundry (in both household and/or commercial laundries) to provide residual self-sanitizing and/or residual microbiostatic (Bacteriostatic and/or fungistatic) activity. This is the reduction in numbers of infectious microorganisms which may contaminate the items) on treated fabrics when used in automatic or manual washing machine operations, usually in the final rinse. Label claims for residual antimicrobial activity on laundered materials or articles can only be considered in those situations when such materials are likely to become and remain damp or wet (for example, diapers and bed linens of incontinent persons)under normal conditions of use and storage between launderings.

(B) Test Procedure – Laundering procedures as described within standard methods such as American Association of Textile Chemists and Colorists (AATCC) 61, AATCC 135, ASTM E 2274 or ASTM 2406 are acceptable for treating the fabric. Alternatively, a simulated-use study utilizing washing machines may be employed. There also are several methods available to evaluate the residual antimicrobial activity of a residual self-sanitizing and/or residual microbiostatic treatment. For example, the basic procedural elements outlined in the "Quantitative Procedure" of the AATCC Test Method 100-2004 "Antibacterial Finishes on Textile Material: Assessment of" or in the ASTM E2149-01 "Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions" may be used to test fabrics for residual activity. *Staphylococcus aureus* ATCC 6538 and *Klebsiella*

pneumoniae ATCC 4352 are acceptable for evaluating the residual antimicrobial activity. Testing should be conducted on three samples representing three different batches of the product, one of which should be at least 60 days old. The following modifications to the methods may be incorporated:

(1) When the control of microorganisms that cause odors are intended, substitute as test microorganisms those target pests that have been identified as the source of the odor problem in wet laundry, and that have been isolated from or are likely to be encountered at the problem site.

(2) If laundered diapers, linens, or other materials which are likely to become soiled with urine are represented as having continued effectiveness in delaying the development of microorganisms that cause odors during storage prior to laundering, *Proteus mirabilis* ATCC 9240 will be required as the test microorganism. If this test microorganism is employed, the swatches must be subjected to a substrate containing urea (for example, urease test medium. The duration (or life expectancy) of the effectiveness of the treatment must be demonstrated.

(3) Calculate the log or percent reduction for each test lot from the geometric mean of the survivors from the test as compared with the untreated parallel control carriers.

(C) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the Test Procedure(s) described in paragraph (vii)(B) of this guideline. Three swatches (carriers) should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1×10^4 colony forming units per carrier.

(D) Additional Organisms – Fungal – The Agency recommends the use of the Test Procedure(s) described in paragraph (vii)(B) of this guideline. Three swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1×10^4 colony forming units per carrier.

(E) Additional Organisms – Viral - The Agency recommends the use of the Test Procedure(s) described in paragraph {vii}{B} of this guideline. Three swatches (carriers) should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1 x 10^4 colony forming units per carrier.

(F) Evaluation of Self sanitizing Laundry Additives and Bacteriostasis Success (residual) – For claims to control odor-causing bacteria, the untreated inoculated control must show bacterial growth. The level of antibacterial activity is indicated by the bacterial reduction attained. For residual bacteriostatic claims against odor-causing bacteria, plate counts of the test microorganism(s) must be less, or no greater than the "zero-time" control and the parallel untreated inoculated control. For residual self-sanitizing claims against odor-causing bacteria, the reduction of each test microorganism must be at least 99.9% over the "zero-time" control and the parallel untreated inoculated control for the time specified (i.e. 24 hours). For residual self-sanitizing claims against pathogenic microorganisms, the reduction of each test microorganism must be at least 99.9% compared to the "zero-time" control and the parallel untreated inoculated control. Parallel tests on the formulation with the active ingredients omitted in an identical manner serve as the control. If such a control solution is not suitable, sterile distilled water to which 0.01percent isooctylphenoxypolyethoxyethanol (9-10 moles oxyethylene, e.g., Triton X-100) may be added.

Fabric and Textile Applications

(i) Fogging of Mattresses and Upholstered Furniture with Gases and Vapors

(A) Test Procedure – Simulated-use studies, in which artificially contaminated articles, e.g., mattresses, upholstered furniture, pillows are employed, must be performed to demonstrate the level of effectiveness intended. The level of effectiveness can be sterilization, disinfection, or sanitization. The studies must be performed in such a way that:

(1) Each test article must be inoculated throughout

the entire article.

(2) Samples must be taken or withdrawn randomly from the entire treated article and cultured for microbial growth.

(3) An adequate control using a similar untreated article must be employed.

(4) The test protocol, including such elements as Replication and test microorganisms will vary with the level of effectiveness intended and the directions for use of the product, but the basic elements described above must be incorporated in any test protocol.

(5) A complete description of the test protocol employed must be submitted, either prior to or with data submission. If the test protocol is not accepted by the EPA, any data generated using the protocol cannot be used in support of a registration.

(6) Testing should be conducted on three samples representing three different batches of the product, one of which should be at least 60 days old.

(B) Evaluation of Application to Mattresses and Upholstered Furniture Success

(1) Sterilization – **Test procedure.** The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants modified for the use direction of the test material to demonstrate the sterilant efficacy of products. Sixty carriers representing each of the two types of surfaces (porcelain penicylinders and silk suture loops), should be tested against the spores of both *B. subtilis* and *C. sporogenes* on one sample of the product. The inoculum employed should provide a count of 1×10^4 - 5×10^6 spores per carrier. Killing on all of the 720 carriers is required. Data submitted to support sterilizing claims will be subject to validation by tests conducted in the Agency's Microbiological Laboratory before the product submitted for registration will be considered acceptable.

(2) Disinfection – **Test procedure.** The Agency recommends the use of the Official Methods of Analysis of AOAC International, Official Method 964.02 Use-Dilution Method or Official Methods of Analysis of AOAC International, Official Method 961.02 Germicidal Spray Products as Disinfectants test modified for the use direction of the test material. Sixty carriers for each of three samples, representing three different batches, one of which should be at least 60 days old, should be tested against each of the following: *Staphylococcus aureus* ATCC 6538 (*S. aureus*), and *Pseudomonas aeruginosa* ATCC 15442 (*P. aeruginosa*). The inoculum employed should provide a count of 1×10^4 - 5×10^6 colony forming units per carrier. The product must kill the test microorganisms on 59 out of each set of 60 carriers.

(3) Sanitization – **Test Procedure.** The Sanitizer Test for Inanimate Non-food Contact Surfaces (prepared by the Registration Division, Office of Pesticide Programs, EPA, 1976) or American Society for Testing and Materials (ASTM) Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (E1153-03) may be used. The propagation of cultures and the use of subculture media and other related equipment may be as specified in Official Methods of Analysis of AOAC International, Chapter 6, Disinfectants. Three product samples, representing three different batches, one of which should be at least 60 days old, should be tested against each test bacterium on each representative test surface depending on the uses proposed on the label. The test microorganisms are: *Staphylococcus aureus* (ATCC 6538) (*S. aureus*) and *Klebsiella pneumoniae* (ATCC 4352) (*K. pneumoniae*) or *Enterobacter aerogenes* (ATCC 13048) (*E. aerogenes*). The results must show a reduction of at least 99.9% in the number of each test microorganism compared to the parallel control count. Calculate the log reduction for each test lot from the geometric mean of the survivors from the test as compared with the untreated parallel control carriers.

(C) Special Note – The use of gases or vapors is currently

the only effective and practical method of treating this type of article.

(ii) Surface Sanitization of Fabrics and Textiles

(A) Test Procedure – The following requirements apply to products which bear claims of sanitization to the surface of fabrics and textiles. The study must be performed using the Non-Food Contact Sanitizer Method with the following modifications:

(1) If a product is intended to be a one-step cleaner/sanitizer, the method must be modified by including an appropriate soil with the bacterial inoculum.

(2) Three product samples representing three separate batches, one of which is at least 60 days old, must be tested against *Staphylococcus aureus* ATCC 6538 and either *Klebsiella pneumoniae* (ATCC 4352) or *Enterobacter aerogenes* ATCC 13048) with 2 different types fabrics. The fabrics chosen must represent natural fabrics, such as cotton, and synthetic fabrics, such as polyester or rayon. If the product is intended for use in hospitals or medical institutions, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). The inoculum employed should provide a count of at least 1×10^4 colony forming units per carrier.

(3) Calculate the log reduction for each test lot from the geometric mean of the survivors from the test as compared with the untreated parallel control carriers.

(B) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the Test Procedure(s) described in paragraph (ii)(A) of this guideline. Five swatches (carriers) for each fabric type should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1×10^4 colony forming units per carrier.

(C) Additional Organisms – Fungal – The Agency recommends the use of the Test Procedure(s) described in paragraph (ii)(A) of this guideline. Five swatches (carriers) for

each fabric type should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1 x 10^4 colony forming units per carrier.

(D) Additional Organisms – Viral - The Agency recommends the use of the Test Procedure(s) described in paragraph (ii)(A) of this guideline. Five swatches (carriers) for each fabric type should be tested against each specific strain for each of two samples representing two different batches. The inoculum employed should provide a count of a minimum of 1 x 10^4 ID_{50} per carrier.

(E) Evaluation of Surface Sanitization of Fabrics and Textiles Success – A 3 log reduction of test organism over the scrubbed control must be demonstrated. Parallel tests on the formulation with the active ingredients omitted in an identical manner serve as the control. If such a control solution is not suitable, sterile distilled water to which 0.01percent isooctylphenoxypolyethoxyethanol (9-10 moles oxyethylene, e.g., Triton X-100) may be added.

(iii) Impregnated Self-sanitizing Fabrics and Textiles

The following requirements apply to products intended for treatment of fabrics and textile materials, usually during the manufacturing process, to provide durable residual self-sanitizing activity (e.g., significant reduction in numbers of infectious microorganisms which may be subsequently deposited on the finished item).

(A)Recommended Test Methods. Residual self-sanitizing products must be evaluated for efficacy using a controlled in-use study or simulated in-use study including the elements outlined below. There are multiple processes to impregnate fabrics and textiles, such as padding, exhaust, or jet application. There also are several methods available to evaluate the residual antimicrobial activity of a self-sanitizing and/or microbiostatic treatment. For example, the basic procedural elements outlined in the "Quantitative Procedure" of the American Association of Textile Chemists and Colorists (AATCC) Test Method 100-2004 "Antibacterial Finishes on Textile Materials: Assessment of" or ASTM E2149-01 "Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions" may be used to test fabric for residual activity. *Staphylococcus aureus* (ATCC 6538) and

Klebsiella pneumoniae (ATCC 4352) are acceptable for evaluating the residual antimicrobial activity. Testing should be conducted on three samples of the fabric/textile representing three different batches of the product, one of which should be at least 60 days old (e.g. 3 textiles/lot).

(B) Test Standard. Each controlled in-use or simulated in-use study must include the following basic elements:

(i) The test microorganisms employed in the study must be pathogens that are likely to be encountered in the environment in which the product is to be used.

(ii) The starting inocula of the test microorganisms (for initial and subsequent challenges) must be of sufficient concentration to provide at least 10^4 survivors on the parallel control surface.

(iii) If necessary, the residue on the treated surfaces must be activated by the addition of moisture in a manner and over an exposure period identical to the use pattern for which the product is intended.

(iv) Quantitative bacteriological sampling must be conducted at frequent and regular intervals. Calculate the log or percent reduction for each test lot from the geometric mean of the survivors from the test as compared with the untreated parallel control carriers.

(v) The same types of fabrics and textiles without the treatment must be employed in the test and inoculated in a manner and over an exposure period identical to the use pattern for which the product is intended.

(vi) The environmental conditions employed in the test (e.g., relative humidity and temperature), must be reported. These conditions must be the same as those likely to be encountered under normal conditions of product use. Tests should also include those environmental conditions that would act to reduce the effectiveness of the treatment (e.g. rinsing, abrasion, organic load, repeated challenges by microorganisms, etc.).

(vii) The length of time the residual activity can be expected to exist under the expected use conditions must be documented.

(C) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the Test Procedure(s) described in paragraph (iii)(A) of this guideline. One swatch (carrier) for each fabric type should be tested

against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1 x 10^4 colony forming units per carrier.

(D) Additional Organisms – Fungal – The Agency recommends the use of the Test Procedure(s) described in paragraph {iii}{A} of this guideline. One swatch (carrier) for each fabric type should be tested against each specific organism for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1 x 10^4 colony forming units per carrier.

(E) Additional Organisms – Viral - The Agency recommends the use of the Test Procedure(s) described in paragraph {iii}{A} of this guideline. One swatch (carrier) for each fabric type should be tested against each specific strain for each of two samples representing two different batches. The inoculum employed should provide a count of a minimum of 1 x 10^4 ID_{50} per carrier.

(F) Evaluation of Application to Impregnated Self-sanitizing Fabrics and Textiles Success – It must be demonstrated that at least 99.9% reduction in the numbers of test microorganisms occurred on the treated surface(s) compared to the parallel control surface(s). For bacteriostatic odor control claims, the numbers of test microorganisms recovered from treated fabrics should be less than the numbers recovered from the parallel control surfaces and no greater then "0-time" control.

Carpet Sanitizers

(i) Carpet Machine Application

(A) Test Procedure – The study must be performed using the proposed method for carpet sanitizers prepared by Registration Division, Office of Pesticide Programs, EPA, 1976; revised 1981. This method, in its entirety, is in section {B} of this guideline. The following conditions must be met:

(1) If a product is intended to be a one-step cleaner/sanitizer, the method must be modified by including at least 5% of an appropriate soil with the bacterial inoculum.

(2) Three product samples representing three separate batches, one of which is at least 60 days old, must be tested against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048) with 2 different types of representative carpeting. If the product is intended for use in hospitals or medical institutions, it must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). All carpet samples tested must be fully identified by the pile fiber, pile yarn weight of finished carpet, pile density, and tuft height. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the test results.

(3) The amount of solution applied to the sample carpeting in the tests must be determined and extrapolated to obtain the amount of the use solution of product to be applied to carpeting (volume per unit area) as stated on the label.

(4) Calculate the log reduction for each test lot from the geometric mean of the survivors from the test as compared with the untreated parallel control carriers.

(B) The proposed method for carpet sanitizers prepared by Registration Division, Office of Pesticide Programs, EPA, 1976; revised 1981 reads:

Special equipment and materials –

1. Carpet mounting board. Mount a piece of 1/8-in. (0.3 cm) tempered hardboard, tempered surface up, on a 16 x 16-in. (40.6 x 40.6 cm) base of ¾-in. (1.8 cm) thick marine plywood, with ¾-in. (1.8 cm) brads.

2. Cutting equipment. 2 x 2-in. (5.1 x 5.1 cm) squares of ¼-in. (0.6 cm) acrylic plastic with 3/32-in. (0.24 cm) holes in the center as templates, and a sharp knife with a replaceable blade.

3. Scrub brushes. 1 ¼ x 3 ½-in. (4.2 x 8.9 cm) surgical hand brush with 5/8-in. (0.6 cm) nylon bristles.

4. Extraction bottles. 8-oz. (236.6 ml), widemouth, round, polypropylene bottles with screw caps (Nalgene 2105 or equivalent) containing 10 stainless steel penicylinders and

100 ml of appropriate neutralizer broth. Similar style glass bottles may be used but care must be taken to prevent breakage during shaking.

5. Spray device. Adjustable spray atomizer modified to feed from a calibrated tube or bottle. A Model 15 DeVilbiss atomizer on a 2-oz. (59.2 ml) bottle graduated with 10-ml marks may be used.

6. Carpet. If the product is intended for use on commercial grade carpeting, 2 representative carpets, such as acrylic and polypropylene tufted-loop type must be tested. No carpeting is available to serve as a standard. If the product is intended for use on wool carpeting, a representative wool sample must additionally be tested. All carpet samples tested must be fully identified, and the pile fiber type, pile yarn weight of finished carpet, pile density and tuft height must be reported. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the test results.

Test cultures and media-

1. Test bacteria. *Use Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). If the product is intended for use in hospitals, *Pseudomonas aeruginosa* (ATCC 15442) must additionally be tested.

2. Nutrient Agar B. AOAC Methods, sec. 4.023 (a)(2).
3. Phosphate buffer dilution water. AOAC Methods, sec. 4023 (f).

4. Double strength neutralizer broth. For phenolic based products, Letheen broth [AOAC Methods, sec. 4.001 (d)(3)] plus an additional 0.7 g lecithin (Azolectin) and 5 g polysorbate 80 (Tween 80) per liter may be used; or a defoaming neutralizer consisting of nutrient broth [AOAC Methods, sec. 4.001 (a)] plus 1.0%Pluronic 25R2 (Meroxapol 252) has been suggested. In the case of halogen or heavy metal based products, 0.1% sodium thioglycollate and 0.01% isooctylphenoxypolyethoxyethanol (Triton X-100) in phosphate buffer (pH 7.2) may be used.

5. Neutralizer plate count agar. Tryptone glucose extract agar [AOAC Methods, sec. 4.037 (a)] plus 0.7 g lecithin (Azolectin) and 5 g polysorbate 80 (Tween 80) per liter.

Bacterial inoculum.
Prepare French square culture bottles with nutrient agar B and test bacteria (AOAC Methods, sec. 4.026). Prepare standardized bacterial stock suspensions by washing growth from bottles and adjust to a density of 10×10^9 bacteria per ml with phosphate buffer dilution water (AOAC Methods, sec. 4.026).

Procedure
1. Cut the carpet into 8 x 12-in. (20.3 x 30.5 cm) pieces. With the aid of the 2 x 2-in. (5.1 x 5.1 cm) template, cut six 2 x 2-in. squares (2 rows of 3 squares per row) from the backing side of the carpet, leaving at least 4 in. (10.2 cm) between the center of each square. The preferred method is to leave about 1/8 in. (0.32 cm) of backing intact at each corner of each cut square so that the entire piece of carpeting can be sterilized and inoculated without separation. Mark the pile surface in the center of each test square with a waterproof marking pen with the aid of the hole in the center of the template. Cover the pile surface of the carpeting with aluminum foil and fold over edges to secure. Steam sterilize and dry. Only carpet that has been determined to be free of residual bateriostatic activity on the pile or backing, following autoclaving, shall be used. A seeded agar plate overlay technique should be used for this determination.

2. Dilute the standardized bacterial stock suspensions, prepared as in (c) above, with phosphate buffer dilution water containing 0.01% isooctylphenoxypolyethoxy ethanol to a concentration of 10×10^7 bacteria per ml. Inoculate the previously marked center of each cut square with 0.1 ml of the bacterial suspension. (Retain the bacterial suspensions for determination of inoculation numbers.) Dry inoculated carpet in an incubator at 35 – 37°C for 60 min. with the foil wrap loosely in place.

3. Condition brushes by immersing the bristles in separate containers (15-cm glass Petri dishes or equivalent) of diluted test solution and a control solution without the active antimicrobial ingredient(s) for 15 min. (If such a control solution is not available, use sterile distilled water containing 0.01% isooctylphenoxypolyethoxyethanol.) Fasten 2 pieces of inoculated carpet (each containing 6 test squares) onto the carpet mounting board by nailing each corner with

upholstery tacks, and with the foil wrapping positioned so as to protect the controls during spraying and scrubbing with the test solution. Place the board in a biological hood or a glove box. A simple safety chamber can be constructed from a large plastic bag.

4. Determine the amount of test solution intended to be applied to one piece of the carpeting containing 6 spots of dried bacterial inoculum [96 sq. in. or 2/3 sq. ft. (244 sq. cm)] and subtract approximately 15 ml which will be applied later in the brushing procedure. Apply the pre-determined amount of diluted test solution at room temperature uniformly by metered spray to one piece of the test carpet. Shake excess test solution from a conditioned brush and transfer to a fresh dish containing 100 ml of test solution at room temperature. Dip bristles of brush and transfer the retained test solution to an inoculated spot on the sprayed carpet. Scrub the spot for 30 sec. using 30 circular clockwise strokes and 30 circular counterclockwise strokes. A circular area of pile approximately 3 in. (7.6 cm) in diameter around each spot must be covered by this treatment. Moderate to heavy pressure should be applied downward on the brush to work the solution to the base of the pile. Repeat dipping of brush into test solution and scrubbing procedure until each of the 6 spots is treated. The brush dipped into the solution no more than 6 times will deliver about 15 ml of solution to the carpet. Do not exceed this amount. Record the total volume of solution applied by spray and brush. Allow the treated carpet piece to remain at room temperature for 60 min. for partial drying of the treated areas.

5. While the piece of carpet treated with the test solution is drying, spray the non-active control solution at room temperature onto half of the other (control) piece of carpet so as to cover 3 of the 6 spots of dried inoculum. Position the aluminum foil over the remainder. Spray an amount equivalent to half of the amount of sprayed test solution. Scrub the 3 wet spots in the same manner as the test carpet. The remaining 3 spots are unscrubbed controls to determine the numbers of bacteria which survived drying of the inoculum. Care must be taken not to wet or scrub over the unscrubbed control area. Allow the scrubbed and unscrubbed controls to remain at room temperature for 60 min. as with the test piece.

6. Following the 70 min. drying periods, cut each 2 x 2-in. test square free with flamed forceps and knife. Transfer each square of carpet to a separate extraction bottle of neutralizer broth. Shake each extraction bottle vigorously for at least 1 min. to free the bacteria from the carpet fibers. Determine the number of viable bacteria in each sample bottle by plating duplicate dilutions in neutralizer plat count agar. Similarly determine the number of viable bacteria in 0.1 ml of the suspension used for inoculating the carpet. Also incubate all broth extraction bottles to determine whether neutralization of the test sample was achieved.

7. Determine the percent reduction of viable bacteria by the test solution by comparing the number of survivors from each treated test square against the average viable count from the scrubbed control squares. An average viable count of at least 1.0×10^6 bacteria from the extracted unscrubbed control squares is necessary for a valid test.

Also see:
Horowitz, William, ed 1975. Official Methods of Analysis of the Association of Official Analytical Chemists, 12th ed. Association of Official Analytical Chemists, Washington, D.C.

(C) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the Test Procedure(s) described in paragraph {i}{A} and {B} of this guideline. One test square for each carpet type should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1×10^6 colony forming units per unscrubbed control square.

(D) Additional Organisms –Reserved.

(E) Additional Organisms –Viral – Reserved.

(F) Evaluation of Carpet Sanitizer (Machine application) Success – A 99.9% reduction of test organism over the scrubbed control must be demonstrated.

(ii) Manual Application

(A) Test Procedure – The same testing should be employed as described above in "Carpet Machine Application" with the following exception:

(1) The method requires that the scrubbing step should be performed using 30 clockwise and 30 counterclockwise strokes, which replicates application using a carpet machine. For manually applied products, the scrubbing step should simulate the product's Directions for Use.

(C) Confirmatory Testing for Additional Organisms – Bacterial – The Agency recommends the use of the Test Procedure(s) described in paragraph (ii)(A) of this guideline. One test square for each carpet type should be tested against each specific bacterium for each of two samples representing two different batches. The inoculum employed should provide a count of at least 1×10^6 colony forming units per unscrubbed control square.

(D) Additional Organisms – Fungal – Reserved.

(E) Additional Organisms – Viral – Reserved.

(B) Evaluation of Carpet Sanitizer (Manual application) Success – A 99.9% reduction of test bacteria over the scrubbed control must be demonstrated.