United States
Environmental Protection
Agency

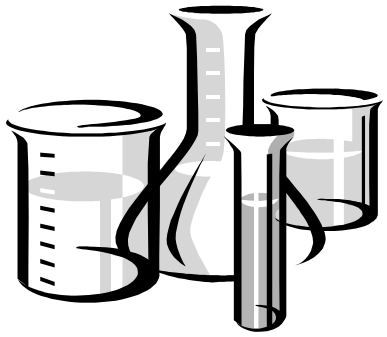
Office of Prevention, Pesticides
And Toxic Substances
(7510P)

EPA 712-C-07-005
Draft November 5,
2009

EPA

Product Performance Test Guidelines

OPPTS 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents



Public Review Draft

NOTICE

This guideline is one of a series of test guidelines established by the Office of Prevention, Pesticides and Toxic Substances (OPPTS), United States Environmental Protection Agency for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and section 408 of the Federal Food, Drug, and Cosmetic (FFDCA) (21 U.S.C. 346a).

The OPPTS test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting the test, and is also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA and/or the FFDCA. As a guidance document, these guidelines are not binding on either EPA or any outside parties, and the EPA may depart from the guidelines where circumstances warrant and without prior notice. The procedures contained in this guideline are strongly recommended for generating the data that are the subject of the guideline, but EPA recognizes that departures may be appropriate in specific situations. You may propose alternatives to the recommendations described in these guidelines, and the Agency will assess them for appropriateness on a case-by-case basis.

For additional information about OPPTS harmonized test guidelines and to access the guidelines electronically, please go to http://www.epa.gov/oppts and select "Test Methods & Guidelines" on the left side navigation menu. You may also access the guidelines in http://www.regulations.gov grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, and EPA-HQ-OPPT-2009-0576.

OPPTS 810.2000: General considerations for public health uses of 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

(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 intends to update these guidelines periodically. However, the use of new methods may be approved, on a case-by-case basis, prior to guideline updates.

(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 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 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 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.

(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 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. 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 products. Since elimination or significant reduction in the number of microorganisms (sterilization, disinfection, sanitization) 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 (inhibition of growth) level are not appropriate for such uses. Bacteriostatic claims are generally only acceptable for products expressly recommended for control of microorganisms of aesthetic significance (e.g., spoilage bacteria, odor-causing bacteria).

(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 or the potential to infect humans. Applicants should consult the Agency for a current listing of organisms which meet these criteria.

(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 kills or effectively reduces the number of living algae in water.

Algistat means any substance, or mixture of substances, that 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 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 fouling of underwater structures or objects.

Antiseptic means a drug product applied topically to the skin 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.

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 conjunction with the term *microbiocide*, are only related to industrial uses.

Biofilm means a community of bacteria or other microorganisms encased in an extracellular polysaccharide substance that attach to a variety of substrates (such as hard surfaces and liquids).

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 destroys or eliminates a specific species of infectious or public health microorganism, but not necessarily bacterial spores, in the inanimate environment.

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, 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.

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.

Slimicide means a substance, or mixture of substances that reduces the number of slime-forming microorganisms in industrial water systems (e.g., paper mills). 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. 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 considerations

(1) Test substance.

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

(ii) Identification should be made of the test substance and quantitative description of its chemical composition should be reported.

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

(iv) The manufacturer 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).

(2) General considerations

(i) Good Laboratory Practice Standards. Antimicrobial products 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.

(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 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 should be accompanied by scientific data that substantiates the antibiotic resistance. The Antibiotic Resistance confirmation 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 organism's growth requirements. The information in paragraphs (e)(3)(i) through (e)(3)(iv) in this guideline should be submitted from the Antibiotic Resistance Confirmation testing.

(i) Test organisms 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 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) is the recommended method.

(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 a component of 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.

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

(ii) The 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).

(iii) The bovine viral diarrhea virus (BVDV) has been adopted as a surrogate for the hepatitis C virus (Ref. 7).

(iv) The feline calicivirus has been adopted as a surrogate for the Noroviruses (Ref.8).

(4) Antimicrobial rinses for fruits and vegetables. To support label claims for consumer-use products as antimicrobial rinses for fruits and vegetables, products 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 fruits and vegetables. 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 peracetic acid containing products, as a method modification to the AOAC Sporicidal Activity Test. (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) should 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 should 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 should 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 should 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) should 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. When the surface to be treated has heavy soil deposits, a cleaning step should be recommended on the label prior to the application of the antimicrobial agent. The effectiveness of antimicrobial agents should be demonstrated in the presence of a specific organic soil at an appropriate concentration 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 solution (e.g., 4.75 mL test solution + 0.25 mL blood serum) before adding 0.5 mL of the test organism.

(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 practical considerations of 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). 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 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.

(h) Microbiological technique considerations

(1) Microorganism survival after drying on a hard surface. Quantitative determinations of the microbial counts on the untreated control carrier after drying should be conducted in order to determine the validity of the test results obtained with the treated carriers. These quantitative determinations should be performed for all carrier-based assays, whether or not modifications are made to the method being used. The test results should include the individual 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 should be conducted in order to determine the level of microbial challenge in the test (Numbers Control). These quantitative determinations should be performed for all suspension assays, whether or not modifications are made to the method being used. The test results should include the individual counts obtained by the recovery method. The detailed final report for suspension testing should 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 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 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.

(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 inoculum is inoculated into the parallel test without the neutralization process to confirm lack of antimicrobial activity of the process (Ref. 15).

(ii) Examples of neutralization techniques: (A) In carrier-based test methods, the carrier is initially deposited in a tube of growth media (i.e., primary subculture). The 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 either through dilution or incorporation of chemical agents in the growth media. A neutralization confirmation for carrier-based test methods may be conducted by demonstrating the growth an inoculum of 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 test. Both the primary cultures and secondary subcultures should be incubated and checked for growth in the test and 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 bioburden 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 be reported for the test and neutralization confirmation in the final report.

(B) A neutralization confirmation for suspension based test methods should be conducted for all neutralization/recovery methods employed in testing. Neutralization confirmation may be conducted by neutralizing the test substance, without the organism, as in the test. Follow by inoculation of a low level of organism (5-100 CFU/mL) and subsequent plating. Plate counts should

be within 1.0 log of a parallel population control.

(C) For virucidal tests, scientifically accepted controls, including proper neutralization controls should 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 product to meet the specified testing or evaluation of success, constitutes evidence that the product is unlikely to be effective as claimed in actual use and is reportable under FIFRA section 6(a)(2).

(i) 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.