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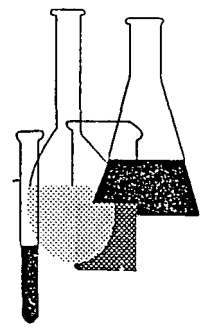
Product Performance Test Guidelines

OPPTS 810.2100 Products for Use on Hard Surfaces—Basic Efficacy Data Requirements



DRAFT

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INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 810.2100 Products for use on hard surfaces—basic efficacy data quirements.

(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.*).

(2) **Background.** The source materials used in developing this harmonized 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) **Sterilizers.** The following information applies to all products represented as sporicidal or sterilizing agents.

(1) **Recommended test methods.** The Association of Official Analytical Chemists (AOAC) Sporicidal Test Method (see paragraph (p)(1) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Sixty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) are required to be tested as outlined in the AOAC Sporicidal Test Method (see paragraph (p)(1) of this guideline) against spores of both *Bacillus subtilis* (American Type Culture Collection (ATCC) 19659) and *Clostridium sporogenes* (ATCC 3584) on three samples representing three different batches of product, one of which is at least 60 days old (240 carriers per sample, or a total of 720 carriers). Any sterilizing agent (vapor or gas) which is recommended for use in a specific device must be tested using the AOAC Sporicidal Test Method in that specific device and according to the directions for use of that specific device.

(3) **Performance standard.** The product must kill the test spores on all of the 720 carriers. No failures are permitted.

(4) **Confirmatory testing.** Confirmatory validation testing should be conducted by a second, independent laboratory of the registrant's choice (other than the laboratory that developed the basic efficacy data).

(i) **Recommended confirmatory test method.** The AOAC Sporicidal Test Method (see paragraph (p)(1) of this guideline).

(ii) **Confirmatory test standard.** Thirty carriers representing each of the two types of surfaces (porcelain penicylinders and silk suture loops), are required to be tested against the spores of both *B. subtilis* and *C. sporogenes* on one sample of the product.

(iii) **Confirmatory performance standard.** The product must kill the test spores on all 120 carriers. No failures are permitted.

(c) **Disinfectants (limited efficacy).** When a disinfectant is recommended in labeling for use against a specific major group of microorganisms (e.g., Gram-negative or Gram-positive bacteria), it is considered to have only limited effectiveness, and consequently, only limited value as a disinfectant. The following requirements apply to such products.

(1) **Recommended test methods—(i) Water-soluble powders and non-volatile liquid products.** The AOAC Use-Dilution Method (see paragraph (p)(2) of this guideline) or the AOAC Hard Surface Carrier Method (distilled water only) (see paragraph (p)(3) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Germicidal spray products (aerosol or pump) and volatile liquid products.** The AOAC Germicidal Spray Products Test (see paragraph (p)(3) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Carriers—60 for each of three samples, representing three different batches, one of which is at least 60 days old, must be tested against *Salmonella cholerasuis* (ATCC 10708) for effectiveness against Gram-negative bacteria, or *Staphylococcus aureus* (ATCC 6538) for effectiveness against Gram-positive bacteria.

(3) **Performance standard.** For the AOAC Use Dilution Method, the product must kill the test microorganisms on 59 out of each set of 60 carriers/slides to provide significance at the 95 percent confidence level. For the AOAC Hard Surface Carrier Method, registrants should contact the Agency for guidance.

(d) **Disinfectants (general or broad spectrum efficacy).** When a disinfectant is represented in labeling as having a broad spectrum of activity (e.g., efficacy against both Gram-negative and Gram-positive bacteria), more extensive testing is required. The following requirements apply to such products.

(1) **Recommended test methods—(i) Water-soluble powders and non-volatile liquid products.** The AOAC Use-Dilution Method (see paragraph (p)(2) of this guideline) or the AOAC Hard Surface Carrier Method (distilled water only) (see paragraph (p)(3) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Germicidal spray products (aerosol or pump) and volatile liquid products**. The AOAC Germicidal Spray Products Test (see paragraph (p)(3) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Sixty carriers for each of three samples, representing three different batches, one of which is at least 60 days old, must be tested against both *S. cholerasuis* (ATCC 10708) and *S. aureus* (ATCC 6538).

(3) **Performance standard.** For the AOAC Use Dilution Method, the product must kill the test microorganisms on 59 out of each set of 60 carriers/slides to provide significance at the 95 percent confidence level. For the AOAC Hard Surface Carrier Method, registrants should contact the Agency for guidance.

(e) **Disinfectants (hospital or medical environment efficacy).** When a product is recommended in labeling for use in hospitals, clinics, dental offices, nursing homes, sickrooms, or any other medical-related facility, the following requirements apply.

(1) **Recommended test methods—(i) Water-soluble powders and non-volatile liquid products.** The AOAC Use-Dilution Method (see paragraph (p)(2) of this guideline) or the AOAC Hard Surface Carrier Method (distilled water only) (see paragraph (p)(3) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Germicidal spray products (aerosol or pump) and volatile liquid products.** The AOAC Germicidal Spray Products Test (see paragraph (p)(4) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Sixty carriers for each of three samples, representing three different batches, one of which is at least 60 days old, must be tested against each of the following: *S. cholerasuis* (ATCC 10708), *S. aureus* (ATCC 6538) *and Pseudomonas aeruginosa* (ATCC 15442).

(3) **Performance standard.** For the AOAC Use Dilution Method, the product must kill the test microorganisms on 59 out of each set of 60 carriers/slides to provide significance at the 95 percent confidence level. For the AOAC Hard Surface Carrier Method, registrants should contact the Agency for guidance.

(f) **Fungicides.** The following requirements apply to non-volatile disinfectants which bear additional label claims of efficacy against fungi pathogenic for man.

(1) **Recommended test methods.** The AOAC Fungicidal Test Method (see paragraph (p)(5) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Two samples representing two different batches of the product must be evaluated for efficacy against *Trichophyton mentagrophytes* (ATCC 9533 is suitable).

(3) **Performance standard.** Killing of all fungal spores is required. The highest dilution that kills all fungal spores is the minimum effective concentration.

(4) **Alternative test methods—(i) Water-soluble powders and non-volatile liquid products.** The AOAC Use-Dilution Method (see paragraph (p)(2) of this guideline). This test may be modified to conform with appropriate elements in the AOAC Funicidal Test Method. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Germicidal spray products (aerosol or pump) and volatile liquid products.** The AOAC Germicidal Spray Products Test (see paragraph (p)(4) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(5) **Alternative test standard.** Ten carriers for each of two samples representing two different batches of the product must be evaluated for efficacy against *T. mentagrophytes* (ATCC 9533 is suitable). The inoculum employed in paragraph (f)(4) of this guideline must be modified to provide a concentration of at least 10^6 conidia per carrier.

(6) **Alternative performance standard.** Killing of the fungal spores on all carriers/slides is required.

(g) **Virucides.** The following requirements apply to disinfectants which bear additional label claims of effectiveness against viruses. Most virucidal products are intended for use on dry inanimate surfaces. For this reason, acceptable virological data are usually developed by carrier methods.

(1) **Recommended test methods.** The Agency will accept adequate data developed by any virological technique which is recognized as technically sound, and which simulates, to the extent possible in the laboratory, the conditions under which the product is intended for use. The specific virus to be tested must be inoculated onto hard, inanimate surfaces (e.g., Petri dishes, glass slides, 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. Each specific virus against which product effectiveness is claimed must be treated. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(i) For virucides whose use-directions identify the product as one intended for use upon dry, inanimate, environmental surfaces (e.g., floors, tables, etc.), carrier methods, which are modifications of either the AOAC Use-Dilution Method (see paragraph (p)(2) of this guideline) for water-soluble powders and non-volatile liquid products or, for products with volatile active ingredients (such as alcohols), the AOAC Germicidal Spray

Products Test Method (see paragraph (p)(4) of this guideline) for germicidal spray products (aerosol or pump) must be used in the development of the virological data.

(ii) To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label.

(iii) 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, must be included with the viral inoculum. Additional organic material need not be incorporated into those procedures where at least 5 percent blood serum is already present in the viral suspension used as the inoculum.

(iv) One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface (e.g., Petri dish, glass slide, cylinder) for a specified exposure period at room temperature.

(v) Following treatment of the test virus with the disinfectant product, the virus is then assayed by an appropriate virological technique. The protocol for the viral assay must provide the following information:

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

(B) Cytotoxicity controls. The effect of the germicide on the viral assay system from a minimum of four determinations per each dilution.

(C) The activity of the germicide against the test virus from a minimum of four determinations per each dilution in the assay system.

(D) Any special methods which are used to increase the virus titer and to detoxify the residual germicide.

(E) The ID50 values calculated for each assay.

(F) The test results must be reported as the reduction of the virus titer by the activity of the germicide (ID50 of the virus control less the ID50 of the test system) expressed as the logarithm to the base 10 and calculated by a statistical method (for example: Reed, L.J., and H. Muench. A simple method of estimating 50 percent endpoints. *American Journal of Hygiene* 27:493–497 (1938); Litchfield, J.T., and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *Journal of Pharmacological Experimental Therapy* 96:99–115 (1949).

(G) For virucidal data to be acceptable, the product must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is

evident (as illustrated in the following tables) at least a 3-log reduction in viral titer must be demonstrated beyond the cytotoxic level. The calculated viral titers must be reported with the test results.

(H) 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 the following tables:

Table 1.—Example of Hypothetical Test Results Demonstrating Virucidal Activity[1]

Dilution inoculated	Virus—Disinfectant[2]	Virus—Control[2]	Cytotoxicity—Control
10^{-1}	TTTT	++++	TTTT
10^{-2}	TTTT	++++	TTTT
10^{-3}	T000	++++	T000
10^{-4}	0000	++++	0000
10^{-5}	0000	++++	0000
10^{-6}	0000	+++0	0000
10^{-7}	0000	+000	0000
10^{-8}	0000	0000	0000

[1] T = toxic; + = virus recovered; 0 = no virus recovered.
[2] Recovery of virus from surfaces demonstrated by cytopathogenic effect, fluorescent antibody, plaque count, animal response, or other recognized acceptable technique.

Table 2.—Calculation of the Tissue Culture Infective Dose 50 percent (TCID50)[1]

Dilution inoculated	number infected/ number inoculated	number infected	number not infected	Accumulated Values			
				number infected	number not infected	number infected/ number inoculated	Percent 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

[1] TCID50 = $10^{6.5}$

Table 3.—Calculation of the Tissue Culture Lethal Dose 50 percent (TCLD50)[1]

Dilution inoculated	number toxic/ number inoculated	number toxic	Accumulated values				
			number not toxic	number toxic	number not toxic	number toxic/ number inoculated	Percent 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

[1] TCLD50 = $10^{2.7}$, therefore, virus inactivation = TCID50 – TCLD50 = $10^{3.8}$ log 10. Claims for virucidal activity for a product must be restricted to those viruses which have actually been tested.

(2) **Test standard.** One surface for each of two samples, representing two different batches of disinfectant, must be tested against a recoverable virus titer of at least 10^4 viable viral particles from the test surface for a specified exposure period at room temperature. (Test surfaces include Petri dishes, glass slides, stainless steel cylinders, etc.) The presence of remaining viable virus following treatment with the product is then assayed by an appropriate virological technique. Separate studies on two batches of product are required to be conducted using each virus against which product efficacy is claimed.

(3) **Performance standard.** Inactivation of virus must be demonstrated at all dilutions when no cytotoxicity is observed in the assay system, or at all dilutions above the cytotoxic level when it is observed. The data must demonstrate at least a 3-log reduction in viral titer for both samples when cytotoxicity is present. The calculated viral titers must be reported with the test results.

(h) **Tuberculocides.** The following requirements, presented in the data call-in notice for tuberculocidal effectiveness data for all antimicrobial pesticides with tuberculocidal claims, dated June 13, 1986 (see paragraph (p)(6) of this guideline), apply to disinfectants which bear additional label claims of effectiveness as tuberculocides. (Note: Certain chemical classes are required to undergo validation testing in addition to basic testing.)

(1) **Recommended test methods.** (i) The AOAC Tuberculocidal Test Method (see paragraph (p)(7) of this guideline) employing standard test conditions of contact time and temperature. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) Tuberculocidal Activity of Disinfectants Test Method with significant modification of the standard test conditions of contact time and/or temperature that are necessary to achieve tuberculocidal efficacy (see para-

graph (p)(6) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(iii) Quantitative Tuberculocidal Activity Test Method. The following test procedure has been published in the Agency's "Data Call-In Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides with Tuberculocidal Claims," dated June 13, 1986 (see paragraph (p)(6) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(A) **Stock culture.** *Mycobacterium bovis* (BCG) TMC 1028 (available from Mycobacterial Culture Collection, National Jewish Hospital and Research Center, Denver, CO) (ATCC 35743). Lyophilized culture of *M. bovis* BCG is inoculated into 10 mL of Modified Proskauer-Beck Medium (refer to paragraph (h)(1)(iii)(C)(*1*) of this guideline) and incubated at 37 °C until a pellicle forms. Transfer a loopful of pellicle onto the surface of 10 mL of Modified Proskauer-Beck Medium with Tween 80 (refer to paragraph (h)(1)(iii)(C)(*2*) of this guideline). Incubate at 37 °C until culture is turbid. Transfer the 10 mL of culture to 100 mL of Modified Proskauer-Beck Medium with Tween 80 (refer to paragraph (h)(1)(iii)(C)(*2*) of this guideline) in a 250-mL flask. Incubate for 5–7 days, shaking the flask daily to aerate. Add 100 mL of culture to a 2-L roller bottle containing 1 L of Modified Proskauer-Beck Medium with Tween 80 (refer to paragraph (g)(1)(iii)(C)(2) of this guideline) and incubate for 15–20 days at 37 °C, rolling slowly. Harvest the cells when the absorbance of the culture, measured at 500 nm, is 0.6 (1-5 $\times 10^8$ CFU/mL). On the day previous to harvesting, add Tween 80 (final concentration = 0.1 percent) to the culture. Homogenize 10–20 mL aliquots of the culture with a Teflon tissue homogenizer in a biosafety hood. Dispense 1–2 mL of the homogenized culture into vials and freeze at –70 °C.

(B) **Test culture.** Remove and thaw a vial of frozen stock culture (refer to paragraph (h)(1)(iii)(A) of this guideline) at room temperature. Add an equal volume of buffered gelatin (refer to paragraph (h)(1)(iii)(C)(*4*) of this guideline) to the cell suspension and homogenize with a Teflon tissue grinder for 1 min, maintaining the culture at 0–4 °C in an ice bath. Dilute the homogenized cell suspension with saline solution (refer to paragraph (h)(1)(iii)(C)(*6*) of this guideline) to approximately 10^7 CFU/mL. To demonstrate minimum culture viability and resistance, the test culture should be tested against phenol solution (refer to paragraph (h)(1)(iii)(C)(*8*) of this guideline) following the procedure described under paragraph (h)(1)(iii)(F) of this guideline. The test culture should show no less than 0.5 $\log_{10}$ and no more than 1.0 $\log_{10}$ kill in 20 min at 25 °C.

(C) **Culture media and solutions.** (*1*) Modified Proskauer-Beck Medium. Dissolve 2.5 g KH_2PO_4, 5.0 g asparagine, 0.6 g $MgSO_4 \cdot 7H_2O$, 2.5 g magnesium citrate and 20.0 mL glycerol in 1 L distilled water. Adjust to pH 7.2–7.4 with 1N NaOH.

(*2*) Modified Proskauer-Beck Medium with Tween 80. Mix 1 mL Tween 80 into 1 L Modified Proskauer-Beck Medium (refer to paragraph (h)(1)(iii)(C)(*1*) of this guideline).

(*3*) Mycobacteria 7H11 Agar. Dissolve 21 g Bacto-Mycobacteria 7H11 agar (Difco) in 1 L distilled water containing 0.5 percent glycerol. Heat to boiling to dissolve medium completely. To each 500 mL sterile medium, cooled to 50–55 °C, add 50 mL of Bacto-Middlebrook OADC enrichment under aseptic conditions. Dispense 15–20 mL into 20×60 mm disposable Petri dishes.

(*4*) Buffered gelatin. Dissolve 2.8 g NaH_2PO_4 in 100 mL of distilled water. Dissolve 5.4 g $Na_2HPO_4 \cdot 7H_2O$ (or 7.2 g $Na_2HPO_4 \cdot 12H_2O$) in 100 mL distilled water. Mix 33 mL NaH_2PO_4 solution with 67 mL Na_2HPO_4 solution and dilute to 200 mL with distilled water (pH 7.1). Dissolve 2.0 g Bacto-gelatin (Difco) in phosphate buffer solution.

(*5*) Saline solution. Add 8.5 g NaCl to 1 L of distilled water, and mix thoroughly.

(*6*) Saline—Tween 80 Solution. Mix 1 mL of Tween 80 in 1 L saline solution (refer to paragraph (h)(1)(iii)(C)(*5*) of this guideline).

(*7*) Phenol stock solution (4 percent). Dissolve 4.0 g of phenol crystals in 100 mL distilled water.

(*8*) Phenol test solution. Make a 1:5 dilution of phenol stock solution. This will result in a final 0.8 percent phenol solution.

(D) **Neutralizer.** A neutralizer appropriate for the active ingredient in the antimicrobial should be used. Static activity controls should be conducted in order to verify neutralizer capability. Also, it may be required to demonstrate that the neutralizer is not active against the test microorganism at concentrations employed in this method.

(E) **Equipment.** (*1*) Glassware, water bath—see AOAC Method 955.11B(a) and (b) (see paragraph (p)(7) of this guideline).

(*2*) Teflon homogenizer—see AOAC Method 966.04B(e) (see paragraph (p)(1) of this guideline).

(*3*) Bacteriologic filters and filter holder—47 mm diameter, 0.45 μm pore size.

(*4*) Roller culture apparatus with 2-L cell culture bottle.

(*5*) Plastic bags—less than 2 mil thickness.

(F) **Procedure.** (*1*) Let 1 tube, containing 9 mL use-dilution germicide sample to be tested, come to the desired temperature of the test in a water bath and add 1 mL of test organism (refer to paragraph

(h)(1)(iii)(B) of this guideline) to the tube containing germicide. Mix by swirling and, at appropriate time intervals, remove aliquots of germicide-cell suspension and add directly to equal volume of appropriate neutralizer (refer to paragraph (h)(1)(iii)(D) of this guideline). Mix thoroughly. Make 10-fold dilutions of neutralized sample in saline (refer to paragraph (h)(1)(iii)(C)(*5*) of this guideline) dilution blanks. Add 10–20 mL of sterile saline to the membrane filter holder fitted with a membrane. Pipette 1 mL of appropriate dilution into the liquid on the surface of the bacteriological filter (refer to paragraph (h)(1)(iii)(E)(*3*) of this guideline). Turn on vacuum to effect filtration. Wash the filter with at least 50 mL of saline with the vacuum on. Remove the filter aseptically from the filter holder and place on the surface of Mycobacteria 7H11 agar (refer to paragraph (h)(1)(iii)(C)(*3*) of this guideline). Incubate the plates in plastic bags (paragraph (h)(1)(iii)(E)(*5*) of this guideline) for 15–20 days at 37 °C. Colonies should be counted using a dissecting microscope and lateral lighting to highlight colonies of *M. bovis* BCG.

(*2*) Survival curves should be constructed to determine the tuberculocidal activity of the solution. Data should be plotted on semilog paper as S/S_0 vs time. S_0 is calculated by determining the viable count of the test organism culture (refer to paragraph (h)(1)(iii)(B) of this guideline) for each replication, and S is the viable count at each time point for each replication. Each ratio for time points should then be averaged to generate data for a survival curve.

(*3*) Survival curves should be the average of at least four separate studies so that upper 95 percent confidence limits can be determined for each point on the curve. The value for each upper 95 percent confidence limit is calculated by multiplying the standard deviation by 1.96. The minimum time that can be claimed for efficacy is determined by finding the point at which the average survival curve intersects the S/S_0 line on the graph which indicates the probability of one survivor (e.g., 1 divided by S_0, the average starting count). This time can also be found by inspection of the raw tabular data, locating the time at which an average of one organism or less was found. If the data show at least 4 $\log_{10}$ kill of the starting population, but the survivor curve does not intersect the one survivor S/S_0 line on the graph, the minimum time that can be claimed is determined by extrapolating the upper 95 percent confidence limit curve to the one survivor line, using the last two points on the 95 percent confidence limit curve as a basis for the extrapolation.

(*4*) The time established for tuberculocidal efficacy claims must be in 5 min increments. In instances where a 0-survivor point or a 95 percent confidence limit intercept leads to a time other than that falling on a 5 min increment, the claim will be established by increasing the time up to the next higher 5 min increment (e.g., if 22 min were a 1-survivor line intercept, the claim time would be 25 min. If an average 0-point were found at 20 min, the claim time would be 20 min). If a contact time of

other than 5 minutes is desired, the testing must be performed by the AOAC Tuberculocidal Activity of Disinfectants Test Method (refer to paragraph (h)(1)(ii) of this guideline), the AOAC Germicidal Spray Products Test or the Presaturated or Impregnated Towelette Test (refer to paragraphs (h)(1)(iv) and (h)(1)(v) of this guideline).

(5) An extrapolation of the upper 95 percent confidence limit interval curve to determine kill time will be considered valid only if the last data point shows at least 99.99 percent (4 $\log_{10}$) kill. The 95 percent confidence limits on the curve should be such that the intersection of the upper 95 percent confidence limit curve with the 1-survivor line (time) should not be at a value that is 50 percent greater than the value where the survivor curve, or an extrapolation from the survivor curve, intersects that line.

(iv) AOAC Germicidal Spray Products Test (see paragraph (p)(4) of this guideline). If the product is a spray or a liquid with volatile active ingredients, the procedure must be modified to conform with the AOAC Germicidal Spray Products Test using the media, test culture, and other elements described in the AOAC Tuberculocidal Activity Method (see paragraph (p)(7) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(v) Presaturated or impregnated towelettes. When the product is packaged as a towelette (particularly when containing volatile active ingredients such as alcohols), the Presaturated or Impregrated Towelettes Simulated Use Test (refer to paragraph (i)(1) of this guideline) must be used with the media, test culture, and other elements as described in the AOAC Tuberculocidal Activity Method (see paragraph (p)(7) of this guideline). Testing must be performed in an open air environment (not a closed petri dish).

(2) **Test standard.** (i) When using the existing or modified AOAC Tuberculocidal Activity Test Method, the AOAC Germicidal Spray Products Test Method, or the Presaturated or Impregnated Towelettes Method, 10 carriers for each of two samples, representing two different batches of product must be tested against *Mycobacterium tuberculosis* var. bovis (BCG).

(ii) When using the Quantitative Tuberculocidal Activity Method, two samples, representing two different batches of product must each be utilized in at least four separate studies (a total of at least eight studies), against *M. tuberculosis* var. bovis, so that upper 95 percent confidence limits can be determined for each point on the survival curve.

(3) **Performance standard.** (i) When using the existing or modified AOAC Tuberculocidal Activity Test Method, the AOAC Germicidal Spray Products Test Method, the Presaturated or Impregnated Towelettes Test Method, killing on all carriers/slides, and no growth in any of the inoculated tubes of two additional media, is required.

(ii) When using the Quantitative Tuberculocidal Activity Method, an extrapolation of the upper 95 percent confidence interval curve to determine kill time will be considered valid only if the last data point shows at least 99.99 percent (4 $\log_{10}$) kill (reduction in the original population number). The 95 percent confidence limits on the curve should be such that the intersection of the upper 95 percent confidence limit curve with the one survivor line (time) should not be at a value that is 50 percent greater than the value where the survivor curve, or an extrapolation from the survivor curve, intersects that line.

(4) **Validation testing requirements for specific chemical classes.** (i) If glutaraldehyde-based products are evaluated using the existing AOAC Tuberculocidal Activity Method or the AOAC Germicidal Spray Products Test Method, employing the standard test conditions of contact time and temperature, validation data will be required. Validation data must be developed by testing one additional sample of the product by a laboratory of the registrant's choice (other than the laboratory which developed the original efficacy data) using the same test conditions as the original laboratory (10 min contact time and 20 °C). If validation testing involves stressed (reused) solutions, consultation with the Agency is advised prior to initiation of testing.

(ii) Products with tuberculocidal claims that are formulated with quaternary ammonium compounds may be evaluated for tuberculocidal efficacy using any one of the test methods listed in paragraph (h)(1) of this guideline. However, validation data is required for any test method chosen. Validation data must be developed by testing one additional sample of the product by a laboratory of the registrant's choice (other than the laboratory which developed the original efficacy data) using the same optional test procedure and test conditions as the original laboratory.

(iii) Products with tuberculocidal claims that are formulated with chemical groups other than glutaraldehyde and quaternary ammonium compounds are permitted, at this time, to be evaluated for tuberculocidal efficacy using any one of the test methods listed in paragraph (h)(1) of this guideline. Validation data does not need to be submitted.

(i) **Presaturated or impregnated towelettes.** Presaturated or impregnated towelettes represent a unique combination of antimicrobial chemicals and applicator prepackaged as a unit in fixed proportions. Therefore, the complete product, as offered for sale, should be tested according to the directions for use to ensure its effectiveness in disinfecting hard surfaces.

(1) **Recommended simulated-use test—(i) Single-use towelettes.** This product is intended to be removed from the package, used immediately, and discarded after use. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(A) The standard test methods available for hard-surface disinfectants (AOAC Use-Dilution Method, the AOAC Hard Surface Carrier Method, and AOAC Germicidal Spray Products Test Method), if followed exactly, would not closely simulate the way in which the disinfectant towelette is used. Of these methods, the AOAC Germicidal Spray Products Test Method appears to be the one most readily modified for this situation. Instead of spraying the inoculated surface of the glass slide, the product should be tested by wiping the surface of the glass slide with the saturated towelette, and then subculturing the slides after the specified holding time. All remaining liquid should be expressed from the used towelette and should also be subcultured.

(B) The towelette should be removed from its container and subsequently handled with sterile gloves. One towelette should be used to wipe 60 inoculated slides. 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 slides. After wiping the last slide for a particular towelette, all of the liquid remaining in the material should be expressed into an empty sterile container by squeezing the towelette; after a specified holding time (equal to the contact time stated on the product label), an aliquot from this container (ca. 0.1 mL) should be subcultured in the same manner as the slides.

(C) For additional test modifications, which may be necessary depending on the intended label claims and directions for use, as well as for documentation of neutralization, refer to OPPTS 810.2000, paragraph (c)(4)—Supplemental recommendations (e.g., exposure period, hard water, organic soil, etc.)

(ii) **Simulated re-use protocol for multiple-use towelettes.** Multiple-use towelettes are 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). Products intended for patterns of repeated use should be tested by a simulated reuse protocol. At the completion of the simulated reuse test, the used towelettes should be tested at the specified end-point of their use-life for effectiveness as disinfectants as indicated in paragraphs (h)(1)(i) and (h)(2)(i) of this guideline. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.) The simulated reuse protocol should include, but is not limited to, the following basic elements:

(A) The cloth should be moistened (in the case of a dry impregnated towelette) and applied to representative types 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 should include periodic challenge with microbiological "bioburden" (viable test bacteria dried onto surfaces/carriers

which are wiped). The minimum bioburden load should be approximately equivalent to one glass slide contaminated with at least 10^6 viable bacteria (e.g., *S. aureus*, *S. cholerasuis*, *P. aeruginosa*) per each 5 mL of use solution produced in wetting the towelette.

(B) Periodic chemical monitoring of the active ingredient in the use solution produced in the cloth should be performed to demonstrate 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 (refer to paragraphs (c), (d), and (e) of this guideline).

(C) For additional test modifications, which may be necessary depending on the intended label claims and directions for use, as well as for documentation of neutralization, refer to OPPTS 810.2000, paragraph (c)(4)—Supplemental recommendations (e.g., exposure period, hard water, organic soil, etc.). If claims are made for the use of the product in the presence of soil (''one-step'' cleaning and disinfecting), the reuse protocol must be conducted with at least 5 percent blood serum added to the bacterial inoculum employed as bioburden as well as to the water.

(D) At the completion of the simulated-use protocol, the used towelettes are tested at the specified end-point of their use-life for effectiveness as disinfectants as indicated in paragraphs (h)(1)(i)(A), (h)(1)(i)(B), and (h)(1)(i)(C) of this guideline.

(2) **Test standard—(i) Single-use towlettes.** Refer to paragraphs (b)(2), (c)(2), and (d)(2) of this guideline for guidance on the test microorganisms and the minimum number of carriers/slides to be used in the simulated-use test for single-use towelettes. Three towelettes from freshly opened packages, representing three different batches of product, one of which is at least 60 days old) should be evaluated for efficacy. Simulated in-use tests are to be conducted in triplicate. No simulated reuse protocol is required.

(ii) **Multiple-use towelettes.** Three towelettes representing three different batches, one of which is at least 60 days old, should be utilized in the simulated re-use protocol. The tests should be conducted in triplicate. The simulated-use test should incorporate, to the extent possible, the conditions under which the product is reused and to which it could be exposed, including repeated microbiological challenge, for the duration of its intended use-life.

(3) **Performance standard—(i) Single-use towelettes.** Refer to paragraphs (b)(3), (c)(3), and (d)(3) of this guideline. Subcultures of the liquid expressed from the used towelettes should be negative for growth.

(ii) **Multiple-use towelettes.** Following reuse testing of the product according to paragraph (h)(1)(ii) of this guideline and subsequent evaluation of the reused product according to paragraph (h)(1)(i) of this guideline, the product should meet the performance standards given in paragraph (h)(3)(i) of this guideline.

(j) **Phenol coefficient.** Data from this test are required only when permitted phenol coefficient claims are made in labeling of disinfectants.

(1) **Recommended test methods.** The AOAC Phenol Coefficient Method (see paragraph (p)(8) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Phenol coefficients for *Salmonella typhi* (ATCC 6539), the only official test organism, and for any other additional Gram-negative or Gram-positive asporogenous bacteria must be determined on each of two samples representing two different batches against each bacterium.

(3) **Performance standard.** The phenol coefficient is a numerical value that compares the bactericidal concentration of a disinfectant to the bactericidal concentration of pure phenol. This numerical value is obtained by dividing the greatest dilution of disinfectant killing *S. typhi*, or other bacterium being evaluated, in ten minutes, but not in five minutes, by the greatest dilution of phenol showing the same results. Since phenol coefficient values are usually an unreliable index as to the effective use-dilution of a disinfectant product, the AOAC Use-Dilution Method must be employed to determine the efficacy of a product, and its effective use-dilution.

(k) **Additional microorganisms.** The following requirements apply to disinfectants which bear label claims against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Hard Surface Carrier Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Test, but not including viruses (see paragraph (g) of this guideline for virucides).

(1) **Recommended test methods—(i) Water-soluble powders and non-volatile liquid products.** The AOAC Use-Dilution Method (see paragraph (p)(2) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Germicidal spray products (aerosol or pump).** The AOAC Germicidal Spray Products Test (see paragraph (p)(4) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(iii) **Presaturated or impregnated towelettes.** Refer to paragraph (i)(1) of this guideline.

(2) **Test standard.** Ten carriers for each of two product samples representing two different batches, must be tested against each additional microorganism.

(3) **Performance standard.** (i) Killing of the test microorganisms on all carriers/slides is required.

(ii) Plate count data, on appropriate culture media, must be submitted on each test microorganism to demonstrate that a concentration of at least 10^4 microorganisms survive the carrier-drying step to provide meaningful results at the 95 percent confidence level (see OPPTS 810.2000, paragraph (c)(4)(vi)).

(l) **Sanitizers—nonfood contact surfaces.** The following requirements apply to product 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) **Recommended test methods.** The Sanitizer Test for Hard, Inanimate Nonfood Contact Surfaces (prepared by Registration Division, Office of Pesticide Programs, EPA, 1976) (see paragraph (p)(9) of this guideline). To substantiate the sanitizing claims for a product, data must be submitted that demonstrate that the product, when used as directed, will substantially reduce the number of test microorganisms on a treated surface over those of an untreated control surface. The following protocol may be used. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(i) Determine the bacterial count in an 18–24 h broth culture and add a 0.01—0.03 mL quantity of the broth culture by spreading on a 1×1 inch square of test surface using a bacteriological loop.

(ii) If the product is intended to be represented as a ''one step'' cleaner-sanitizer, an appropriate organic soil load, such as 5 percent blood serum, must be added to the bacterial inoculum.

(iii) The square of test surface should be dried for 40 min in a bacteriological incubator at 30–37 °C.

(iv) A ''zero-time'' bacterial numbers recovery test must be performed to demonstrate the efficiency of the recovery process and must be reported. The ''zero-time'' test shall show the loss in viability that occurred during carrier drying.

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

(vi) 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).

(vii) After a suitable time interval, recover the test organisms by washing the squares with adequate agitation in appropriate media or dilution fluid containing appropriate neutralizers.

(viii) Make plate counts on appropriate nutrient agar containing the same neutralizers by the pour or spread plate technique.

(ix) Exposure time intervals between 0-time and 5 min must be tested for the product as well as the untreated controls.

(x) The results must demonstrate a bacterial reduction of at least 99.9 percent over the parallel control within 5 min.

(2) **Test standard.** The propagation of cultures specified in this paragraph, and the use of subculture media and other related equipment may be as specified in Chapter 6, Disinfectants, of the Official Methods of Analysis of the Association of Official Analytical Chemists. Three product samples, representing three different batches, one of which is 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: *S. aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). *Enterobacter aerogenes* (ATCC 13048) can be substituted for *K. pneumoniae*. The test surfaces representing the types of surfaces recommended for treatment on the label include, but are not limited to, glass, metal, unglazed or glazed ceramic tile, porcelain, or vitreous china.

(3) **Performance standard.** The results must demonstrate a reduction of at least 99.9 percent (a 3-log reduction) in the number of each test microorganism over the parallel control count within 5 min.

(m) **Sanitizing rinses for previously cleaned food-contact surfaces.** The following requirements apply to any product 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. Antimicrobial agents applied to food-contact surfaces are defined as incidental food additives under the Federal Food, Drug, and Cosmetic Act, as amended (21 U.S.C. 201 *et seq.*) and require a food additive tolerance or an exemption from such tolerance according to 21 CFR 178.1010. Recommendation of a potable water rinse after treatment is not permitted for products intended for use as a terminal sanitizing rinse.

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

(i) **Recommended test methods.** The AOAC Available Chlorine Germicidal Equivalent Concentration Method (see paragraph (p)(10) of this

guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Test standard.** Three samples, representing three different batches, one of which is at least 60 days old, must be evaluated for efficacy against *S. typhi* (ATCC 6539). When claims are made for the effectiveness of the product in hard water, all required data must be developed at the hard water tolerance claimed. (See OPPTS 810.2000, paragraph (c)(4)(iii).)

(iii) **Performance standard.** Test results must demonstrate product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

(2) **Other chemical products.** Sanitizing rinses formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations.

(i) **Recommended test methods.** The AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method (see paragraph (p)(11) of this guideline). (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(ii) **Test standard.** Three samples, representing three different batches, one of which is at least 60 days old, must be evaluated for efficacy against both *Escherichia coli* (ATCC 11229) and *S. aureus* (ATCC 6538). When claims are made for the effectiveness of the product in hard water, all required data must be developed at the hard water tolerance claimed. (See OPPTS 810.2000, paragraph (c)(4)(iii).)

(iii) **Performance standard.** Acceptable results must demonstrate a 99.999 percent reduction in the number of each test microorganism within 30 sec. The results must be reported according to the actual count and percentage reduction over the control. The minimum concentration of the product which provides the results required is the minimum effective concentration.

(n) **Residual bacteriostatic activity of dried chemical residues on hard inanimate surfaces.** Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria) in the presence of moisture, but not against microorganisms of public health concern. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(o) **Residual self-sanitizing activity of dried chemical residues on hard-inanimate surfaces.** The following requirements apply to 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) **Recommended test methods.** Residual self-sanitizing products for use on hard, inanimate surfaces must be evaluated for efficacy using a controlled in-use study or simulated in-use study including the elements outlined under paragraph (o)(2) of this guideline. (Refer to OPPTS 810.2000 for general testing requirements prior to initiating product testing.)

(2) **Test standard.** Each controlled in-use or simulated in-use study test 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) 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.

(v) The same types of surfaces 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 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 be documented.

(3) **Performance standard.** For residual self-sanitizing claims, it must be demonstrated that a product is capable of reducing the number of test microorganisms on the test surface by 99.9 percent over that of the parallel control surfaces.

(p) **References.** The following references may be consulted for additional background information.

(1) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Official Method 966.04 Sporicidal Activity of Disinfectants. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(2) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Use-Dilution Methods, Chapter 6, Disinfectants. Fifteenth edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(3) *Official Methods of Analysis of; the Association of Official Analytical Chemists*, Hard Surface Carrier Methods, Chapter 6, Disinfectants. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(4) *Official Methods of Analysis of the Association of Official Analytical Chemists* , Official Method 961.02 Germicidal Spray Products as Disinfectants. Fifteenth edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(5) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Official Method 955.17 Fungicidal Activity of Disinfectants. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(6) 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).

(7) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Official Method 965.12 Tuberculocidal Activity of Disinfectants. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(8) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Official Method 955.11 Testing Disinfectants Against Salmonella typhi. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

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

(10) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Official Method 955.16 Chlorine Equivalent Concentration. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.

(11) *Official Methods of Analysis of the Association of Official Analytical Chemists*, Official Method 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants. Current edition. Association of Official Analytical Chemists (AOAC), 2200 Wilson Boulevard, Arlington, VA 22201.