Agriculture Canada

October 31, 1980 T-1-215

FOOD PRODUCTION AND INSPECTION BRANCH	DIRECTION GENERALE, PRODUCTION ET INSPECTION DES ALIMENTS	SECTION PESTICIDES

TRADE MEMORANDUM

RE: Efficacy Data for Antimicrobial Products

The purpose of this Memorandum is to inform registrants of the efficacy data which will be required in support of the registration of antimicrobial products. For information on requirements for new antimicrobial active ingredients, consult Trade Memorandum T-1-223 and the Registration Guidelines.

Section 4 of the Pest Control Products Act requires that antimicrobial products imported into or sold in Canada must be registered. Exemptions to this requirement are listed in Section 3 of the Regulations under the Pest Control Products Act and are described in Trade Memorandum T-1-214.

For purposes of the Pest Control Products Act, an antimicrobial product is defined as any product used for the control of bacteria, fungi, viruses or other microbes in or on inanimate objects and the environment except food or feed. The term "antimicrobial product" includes such subcategories as:

1. Disinfectants (including sterilizers, germicides, bactericides, sporicides, fungicides, and virucides);

2. Sanitizers;

3. Slimicides;

4. Microstatic agents.

When labels for antimicrobial products are submitted as requested in the Pest Control Products general "Registration Guidelines", antimicrobial label claims subject to regulation under the Pest Control Products Act must be supported by efficacy data as outlined in the attached "Guidelines for Registering Antimicrobial Products".

Submission of the above information for each product will allow the earliest possible review of the data and label and provide an opportunity for the development of any additional data that may be required.

Four bound copies of all submitted information should be sent to the following address:

Pesticides Section
Plant Products and Quarantine Division
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Distribution: PPD-2; PCP-1,2,3,4,5,8,10,12

GUIDELINES FOR REGISTERING ANTIMICROBIAL PRODUCTS

These guidelines are primarily concerned with the efficacy data requirements to support claims made in labelling. In addition, copies of enclosures that are frequently provided to registrants by the Environmental Protection Agency and pertinent references relating to testing of such products are included.

These guidelines are subject to change. Continuous efforts are being made to devise new methods, improve existing ones through collaborative testing and to involve a greater number of persons and firms in the program to develop test methods for adequately evaluating the efficacy of products. The applicant should assure himself that the test method selected to substantiate efficacy is current and applicable to the product proposed for registration. For purposes of identification, where reference is made to test codes in this Appendix, see Table I under "Tests for Efficacy".

Efficacy data requirements for registration are designed to provide:

1. Some evidence that the applicant can reproduce the product formula so that it will have the same level of activity.

2. Evidence that the formula which is being offered for sale will have reasonable shelf-life.

3. A sufficient volume of testing so that deficiciencies which may be subsequently found in the Laboratory Services Division cannot be ascribed to variations in testing which may be encountered among different laboratories.

Reference to "preparation" in the requirements below means individually formulated preparations of the product, either in the laboratory or from production.

Data Requirements

Minimal Disinfectant Claims

Minimal disinfectant claims must be substantiated with efficacy data derived from either the AOAC Use Dilution Method (liquid products) or the AOAC Germicidal Spray Products Method (spray products). Required are sixty replicates on each of three samples, representing three different preparations against Salmonella choleraesuis. One of these preparations aged to represent a 60-day shelf-life stability study must also be tested with 30 replicates on duplicate samples against S. choleraesuis. See Test D-01.010R.70 for Use-Dilution Method and Test D-10.060R-70 for Germicidal Spray Products Method.

General Disinfectant Claims

General disinfection claims are much broader than minimal claims and must be substantiated with the efficacy data required for minimal claims, along with efficacy data derived from two other test species. Sixty replicates are required on each of three samples, representing three different preparation, against S. choleraesuis. In addition thirty replicates on each of these three samples against Staphylococcus aureus and one other pathogen exclusive of S. aureus and S. choleraesuis must be tested. One of these preparations aged to represent a 60-day shelf-life stability study must also be tested with 30 replicates on duplicate samples against S. choleraesuis. See Test D-01.010R-70 for Use Dilution Method and Test D-01.060R-70 for Germicidal Spray Products Method.

Phenol Coefficient Claims

Any phenol coefficient claim requires testing by the AOAC Phenol Coefficient Method in duplicate on one preparation against Salmonella typhosa. Where a phenol coefficient is declared against any other bacterium the test also must be run against each bacterium named. See Test D-01.020R-70, Phenol Coefficient Method.

Claims Against Pathogenic Fungi

Claims against specific pathogenic fungi must be supported with efficacy data derived from duplicate samples of one preparation using the AOAC Fungicidal Test. See Test D-01.030R-70.

Claims against Mycobacterium tuberculosis

Claims against M. tuberculosis must be substantiated with efficacy data derived from the AOAC Tuberculocidal Activity Method (Test D-01.040R-70) on one preparation of the product (liquid) under test. If the product is a spray, the procedure must be modified to conform with the AOAC Germicidal Spray Product Method (Test D-01.060R-70) using the media, microorganisms, etc., described in the AOAC Tuberculocidal Activity Method.

Disinfectant Claims Against Other Microorganisms

Claims for effectiveness against specific microorganisms other than those named in the AOAC Use Dilution Method (Test D-01.010R-70) or the AOAC Germicidal Spray Products Method (Test D-01.060R-70) can be accepted only when supported by efficacy data derived from ten replicates on one preparation against each microorganism claimed.

Sporicidal Claims

All sporicidal claims must be substantiated with efficacy data derived from the AOAC Sporicidal Test (Test D-04.010R-70). The test requires thirty replicates on each of two kinds of carriers (porcelain penicylinders and surgical silk suture loops) against spores of both Bacillus subtilis and Clostridium sporogenes on three different preparations (total of 120 carriers

on each preparation). Thirty carriers of each type must also be tested against spores of either microorganism on duplicate samples of ones of the three preparations, aged to represent a 60-day shelf-life stability (total of 120 carriers for the stability test). Added claims for effectiveness against spore-forming organisms other than B. subtilis and C. sporogenes must be substantiated with data derived from 30 replicates (porcelain penicylinders) on one preparation.

Sterilizing Claims

The AOAC Sporicidal Test (Test D-05.010R-70 and Enclosure DIS-12) is used as the basis for substantiating sterilizing claims. Since "sterilizer" is an absolute term (to destroy all living things), the data requirements supporting a sterilizing claim are greater than those required to support a sporicidal claim. The basic data (360 carriers) called for under "Sporicidal Claims", apart from the 60-day shelf-life study, is also required for sterilizing claims.

In addition, 30 carriers of each type must be tested against spores of B. subtilis and C. sporogenes on duplicate samples of one preparation, aged to represent a 60-day shelf-life stability study (total of 240 carriers, plus 360 carriers equals 600 carriers). No growth must be observed in any of the subculture or resubculture tubes from the 600 carriers.

Virucidal Claims

To support virucidal claims, the registrant is referred to enclosure DIS-13, entitled "Methods for Evaluating the Virucidal Activity of Disinfectants" (Test D-06-010S-72).

Sanitizing Rinses

Sanitizing rinse claims for iodophors, mixed halides and chlorine-bearing chemicals must be substantiated with efficacy data derived by the Available Chlorine Germicidal Equivalent Concentration Method (Test D-03.020R-70). Data from one test on each of three samples representing three different preparations, showing the concentrations of the product equivalent in activity to the reference standards of 50, 100 and 200 ppm of available chlorine as sodium hypochlorite, against S. typhosa are required. Data from duplicate samples of one or these preparations, representing a 60-day shelf-life stability study, showing concentrations of the product equivalent in activity to the three reference standards must be furnished. Chemical analyses may be substituted for biological testing on the 60-day sample.

Sanitizing rinse claims for quaternary ammonium compounds, chlorinated trisodium phosphate and anionic detergent-acid formulations must be substantiated with efficacy data derived by the Germicidal and Detergent Sanitizers Method (Test D-03.010R-70}. Data from one test on each of three different preparations against both E. coli and S. aureus are required, along with data from duplicate samples of one of these preparations representing a 60-day shelf-life stability study against E. coli. When claims for the effectiveness of the product in hard water are made, data derived from test on

duplicate samples of one preparation against both E. coli and S. aureus are required at the hard water tolerance claimed.

In testing the efficacy of detergent sanitizers, it may be necessary to modify the AOAC standardized tests to include appropriate neutralizers that are not specified in the methods. For example, in testing chlorinated trisodium phosphate, sodium thiosulfate is required to neutralize the residual chlorine.

Laundry Additives

Residual bacteriostatic claims for laundry additives must be substantiated with efficacy data derived from the AOAC Bacteriostatic Activity of Laundry Additives Method (Test D.02.010R072). Three samples representing three product preparations are tested, with five 1-inch square treated swatches against S. aureus and Klebsiella pneumoniae, aberrant, ATCC 4352 on seeded agar for each sample. Duplicate samples of one preparation representing 60-day shelf-life stability must be tested with five replicate fabric swatches on agar seeded with S. aureus.

For products bearing sanitizing claims, the method of Petrocci and Clarke may be employed to provide efficacy data to support registration (See Test D-03.070T-69), since no official test method is available. Tests may be carried out in a washing machine in lieu of the Petrocci and Clarke method. The test organisms are K. pneumoniae, ATCC 4352 and S. aureus, ATCC 6558. General protocols, propagation of cultures, fabric to water ratios, test materials, water temperatures and subculture media must be the same as specified in Test D-03.070T-69. Tests must be conducted with three samples representing three product preparations, each sample tested with three replicate fabric swatches against the two test bacteria specified. One sample representing a 60-day shelf-life storage must be tested in duplicate against S. aureus. A 99.9% reduction within 10 minutes must be demonstrated over the control counts.

To support disinfecting claims, the Petrocci and Clarke method (Test D-03.070T-69) is employed; however, the tests are conducted with three samples representing three product preparations, each sample tested with 30 replicate fabric swatches against the two test bacteria specified. One sample representing 60-day shelf-life storage must be tested in duplicate with 15 swatches against S. aureus. The method must be modified to include testing of both the fabric and the wash water (5 ml from the automatic washer or 0.5 ml from the container with the test device) in individual wide-mouth jars containing subculture media and neutralizers. The added wash water to media volume ratio should not exceed 1:40. Growth or no growth is noted after 48 hours incubation. The data requirements outlined above for laundry additives do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones or trichloro-s-triazinetrione.

Textile Preservatives

Methods prescribed by the American Association of Textile Colorists and Chemists involving agar plates (see Test D-02.020S-65) or liquid culture media (see Test D-02.030S-65) may be employed to support residual bacteriostatic claims.

Air Sanitizers

No standard method of evaluating air sanitizer claims has been adopted. Reports by Stuart and Friedl, by McGray, and the British Standards Institution describe methods of testing aerosol products designed for the temporary sanitization of air spaces. Several investigators have shown that the incorporation of glycols (triethylene, propylene or dipropylene glycol) in pressurized formulations at concentrations of 5 to 10 percent cause significant reductions of air-borne bacteria when adequate vapor concentrations are achieved. Currently, since no standard method of evaluating such products is available, registration requirements regarding efficacy are satisfied by chemical analysis data showing appropriate concentrations of glycols. Air sanitizing products that do not contain such concentrations of glycols must be evaluated by a room or chamber technique; a significant reduction of air-borne microorganisms must be demonstrated. The primary test organism is S. aureus. Depending on the intended use pattern, other organisms such as Salmonella or Pseudomonas, may also be used to provide efficacy data.

Carpet Sanitizers

No final official method exists for testing the efficacy of carpet sanitizers; however, a tentative method has been developed through the efforts of the AOAC and is delineated under the tests listed later in this section of the Appendix (see Test D-03.040T-71). If this test is employed to derive data to support the registration of a carpet sanitizer, the following protocols must be considered:

a. Three product samples representing three separate preparations must be tested against each of the two bacteria specified in the method with two different types of carpet. A 99.9% reduction of test bacteria over the control count must be demonstrated in each case. If the product is intended for use on commercial-grade carpeting, two representative carpets such as "acrylic" and "polypropylene" tufted-loop type may be tested. No carpeting is available to serve as a standard. If the product is intended for use on wool carpeting, an additional representative sample must be tested. If not, the label must bear a disclaimer for such use. All carpet samples tested must be fully identified, and the pile fiber type, pile yarn weight of finished carpet, pile density and tuft height reported. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the test results.

b. One sample preparation representing a 60-day shelf-life stability study must be tested with one carpet sample and one test bacterium.

The amount of sanitizer applied to a piece of carpet must be extrapolated to gallons of diluted shampoo to square feet of carpet and this must be declared on the proposed label. We would expect this to be 12 gallons of dilute product to 800 to 1200 square feet of carpet.

Swimming Pool

Swimming pool water disinfectant claims must be substantiated with efficacy data derived from the AOAC Swimming Pool Water Disinfectants Method (Test D-01.050R-70). Data from one test on each of three different preparations against both E. coli and Streptococcus faecalis are required. Data from duplicate samples of one preparation representing a 60-day shelf-life stability study against B. coli must be submitted.

The data requirements outlined above for swimming pool water disinfection do not apply to simple formulations of sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones, or trichloro-s-triazinetrione when adequate directions for pool water maintenance are provided on the product label.

Miscellaneous Products

Adequate tests have been developed for demonstrating efficacy of a few products that do not logically fall into the above categories. Included are methods for testing antimicrobial products to control the deterioration of paints or to control the contamination of hydrocarbon/water systems in the fuel industry. Details of the methods are provided in publications from the American Society for Testing and Materials, the American Petroleum Institute or the Society for Industrial Microbiology; references to the published methods are given on page 8.

Tests for Efficacy

The following list of tests (Table 1) are used to support the claims for various disinfectant-type products. The test code number indicates the broad category of products considered in these guidelines ("D" used with disinfectant type products), the specific product (01. = disinfectants; 02. - bacteriostats; 03. = sanitizers; 04. = sporicides; 05. = sterilizers; 06. = virucides; 07. = miscellaneous types not classified otherwise), the status of the test (R = required, official test; T = tentative test, usually under collaborative study; S = suggested, unofficial test not yet developed to the state requiring collaborative study), and "65", "69", "70", etc., indicating the most recent date associated with the development of the test.

Complete references to cited published tests are provided at the end of this section. Tentative or suggested tests, usually provided as enclosures by microbiologists of the Pesticides Regulation Division, Environmental Protection Agency, are also provided at the end of this document.

TABLE 1. Methods for Testing Efficacy of Disinfectant Type Products

Test Code Number	Product or Claim	Method
D-01.000	Disinfectants	
D-01.010R-70	Minimal disinfectant claims	AOAC Use Dilution
D-01.010R-70	General disinfectant claims	AOAC Use Dilution
D-01.020R-70	Phenol coefficient claims	AOAC Phenol Coefficient
D-01.030R-70	Pathogenic fungi claim	AOAC Fungicidal Test
D-01.040R-70	Tuberculosis claim	AOAC Tuberculocidal Activity
D-01.010R-70	Other microorganisms claim	AOAC Use Dilution
D-01.050R-70	For swimming pools	AOAC Water Disinfectants for Swimming Pools
D-01.060R-70	Spray products	AOAC Germicidal Spray Products
D-01.070T-69	Laundry wash water or fabric	Petrocci and Clarke
D-02.000	Bacteriostats	
D-02.010R-12	For laundry	AOAC Bacteriostatic Activity of Laundry Additives
D-02.020S-65	For fabrics	AATCC Test 90-1965
D-02.030S-65	For fabrics	AATCC Test 100-1965
D-03.000	Sanitizers	
D-03.010R-70	Quaternary ammonium or detergent-acid formulations	AOAC Germicidal and Detergent Sanitizers
D-03.020R-70	Iodophors, chlorine and mixed halide products	AOAC Available Chlorine Germicidal Equivalent Conc.
D-03.010R-70	Hard water claims	AOAC Germicidal and detergent sanitizers
D-03.030	Air sanitizers	Simulated In-Use
D-03.040T-71	For carpets	PRD Enclosure DIS-15
D-03.050T-71	For non-food contact surfaces	PRD Enclosure DIS-5
D-03.060T-72	For residues on hard surfaces	PRD Enclosure DIS-14
D-03.070T-69	For laundry wash water or	Petrocci and Clarke fabric

TABLE 1. (cont.) Methods for Testing Efficacy of Disinfectant Type Products

Test Code Number	Product or Claim	Method
D-04.000	Sporicides	
D-04.010R-70	Sporicide products	AOAC Sporicidal Test
D-05.000	Sterilizers	
D-05.010R-70	Sterilizers (chemical)	AOAC Sporicide Test and PRD Enclosure DIS-12
D-06.000	Virucides	PRD Enclosure DIS-13
D-07.000	Miscellaneous	
D-07.010R	Oil well subsurface injection water additives	American Petroleum Institute
D-07.020T-67	In can paint preservative	ASTM Test D-2574-67T
D-07.030S-66	Antimicrobial fuel additives	Proposed procedure of the Society for Industrial Microbiology

Standard Enclosures

Various standard enclosures have been prepared by the Environmental Protection Agency for distribution to registrants. Copies of the enclosures are reprinted in the following pages; the titles and numbers are as follows:

Title	Code Number
Efficacy Data Requirements - I	DIS-1
Efficacy Data Requirements - II	DIS-2
Efficacy Data Requirements - III	DIS-3
Special Directions for Poultry House Disinfectants	DIS-4
Sanitizer Test (for inanimate, non-food contact surfaces)	DIS-5
Bacteriostats, Sanitizers and Disinfectants Used for Laundering Textiles	DIS-6
Duplicated Product Formulations Based on Data Provided by Basic Manufacturers	DIS-7
Product Formulations Identical to Registered Products	DIS-8
Formulations Produced by Simple Aqueous Dilutions of Registered Concentrates	DIS-9
Tentative Test Method for Laboratory Evaluation of Rug and Carpet Sanitizers or Bacteriostats	DIS-10
Special Directions for Cresylic Acid and Synthetic Phenols (For Use as Farm Premise Disinfectants which Permit Classification as Non-Food Use)	DIS-11
Efficacy Data Requirements for Sterilizing Claim	DIS-12
Methods for Evaluating the Virucidal Activity of Disinfectants	DIS-13
Tentative Residual (Self-Sanitizing) Method	DIS-14

Efficacy Data Requirements - I

A. For Disinfectants (Minimal or general claims):

A.O.A.C. Use Dilution Method; ( ) A.O.A.C. Germicidal Spray Method

Sixty replicates on each of three samples, representing three different preparations against: ( ) Staphylococcus aureus, ( ) Salmonella choleraesuis.

Thirty replicates on each of three samples, representing three different preparations against:
( ) Staphylococcus aureus, ( ) Salmonella choleraesuis,
( ) Pseudomonas aeruginosa, ( ) Trichophyton interdigitale,
( ) One pathogen (exclusive of S. aureus and S. choleraesuis).

Thirty replicates each on duplicate samples of one preparation, representing a 60-day shelf-life stability study against:
( ) S. aureus, ( ) S. choleraesuis.

Ten replicates on one sample against each other pathogen claimed.

B. Phenol Coefficient Data:

A.O.A.C. Phenol Coefficient Tests in duplicate on one sample of one preparation against:
( ) Salmonella typhosa, ( ) S. aureus, ( ) all other pathogens claimed.

C. Fungicidal, Tuberculocidal, or Virucidal Tests:

A.O.A.C. Fungicidal Test on duplicate samples of one preparation.

A.O.A.C. Tuberculocidal Test on one preparation (one sample).

A.O.A.C. Tuberculocidal Test Modified to conform with the A.O.A.C. Germicidal Spray Method, on one preparation (one sample).

Acceptable Virological Techniques. Data on one sample in duplicate against all specific viruses named on the label.

NOTE: It should be understood that where test organisms other than those specified in the Methods outlined above are substituted therein, the media, incubation time, and other factors will all be evaluated in terms of the significance of the results claimed.

Reference: A.O.A.C. Official Methods of Analysis, 11th Ed., Chapter 4 - Disinfectants, pp. 59 - 74, 1970.

Efficacy Data Requirements - II

D. Seeded Agar Plate Test. Procedure as in AATCC 90-1965, B 175-176, 1966, Technical Manual of American Association of Textile Cheists and Colorists, Vol. 40, 1968. (Modified to replace fabric carrier with appropriate hard surface carrier such as steel or ceramics.)

( ) Three samples representing three different preparations of formula or material. Five 1-inch square samples of material or material treated as directed with formula using ( ) S. aureus, ( ) E. coli.

( ) Duplicate samples of one preparation following 60-day storage stability study employing five 1-sq. in. samples using S. aureus.

E. A.O.A.C. Germicidal and Detergent Sanitizer Test:

( ) Three samples representing three different preparations each against both Escherichia coli and Staphylococcus aureus.

( ) Duplicate samples of one preparation representing a 60-day shelf-life stability study, each against E. coli.

Note: If hard water uses are indicated, the above tests must be conducted in the hard water at the tolerances claimed instead of distilled water.

F. A.O.A.C. Available Chlorine Germicidal Equivalent Concentration Test:

( ) Three samples representing three different preparations, showing the concentrations of the formulation equivalent in activity to the three reference standards of 50, 100, and 200 ppm av. C1. as sodium hypochlorite, using Salmonella typhosa.

( ) Duplicate samples of one preparation, representing a 60-day shelf-life stability study*, showing the concentrations of the formulation equivalent in activity to the three reference standards.

*Chemical analysis may be substituted for biological testing for organic chlorine-bearing chemicals.

NOTE: It should be understood that where test organisms other than those specified in the Methods outlined above are substituted therein, the media, incubation time, and other factors will all be evaluated in terms of the significance of the results claimed.

Reference: A.O A.C. Official Methods of Analysis, 11th Ed., Chapter 4 - Disinfectants, pp 59 - 74, 1970.

Efficacy Data Requirements - III

G. A.O.A.C. Swimming Pool Water Disinfectant Method:

( ) Three samples representing three different preparations against Escherichia coli and Streptococcus faecalis.

( ) Duplicate samples of one preparation representing a 60-day shelf-life stability study against Escherichia coli.

( ) Field Tests in Swimming Pools as prescribed in NSF Standards, showing no more than 15% of samples exceed standard plate count of 200 colonies per ml., 2.2 coliforms per 100 ml., or 2.2 enterococci per 100 ml. (National Sanitation Foundation, P.O. Box 1468, Ann Arbor, Michigan 48106.)

H. A.O.A.C. Sporicidal Test:

( ) Three samples representing three different preparations using: both types of carriers (porcelain penicylinders and surgical silk suture loops): both test organisms (Bacillus subtilis and Clostridium sporogenes); thirty replicates with each organism, on each type of carrier (Total of 120 replicates or carriers).

( ) Duplicate samples of one preparation representing a 60-day shelf-life stability study using: 30 replicates with one test organism on both types of carriers.

( ) Duplicate samples of one preparation representing a 60-day shelf-life stability study using: 30 replicates with both test organisms named above on both types of carriers. (Total of 120 replicates or carriers for each sample.)

( ) One sample of one preparation using 30 replicates (porcelain penicylinders) for each spore-forming organism named other than B. subtilis and C1. sporogenes.

NOTE: It should be understood that where test organisms other than those specified in the Methods outlined above are substituted therein, the media, incubation time, and other factors will all be evaluated in terms of the significance of the results claimed.

Reference: A.O.A.C. Official Methods of Analysis, 11th Ed., Chapter 4 - Disinfectants, pp 59 - 74, 1970.

Special Directions for Poultry House Disinfectants

1. Remove all poultry and feeds from premises, trucks, coops, crates.

2. Remove all litter and droppings from floors, walls, and surfaces of facilities occupied or traversed by poultry.

3. Empty all troughs, racks, and other feeding and watering appliances.

4. Saturate surfaces with recommended solution.

5. Ventilate buildings, coops and other closed spaces. Do not house poultry or employ equipment until treatment has been absorbed, set, or dried.

6. All treated feed racks, mangers, troughs, automatic feeders, fountains, and waterers must be thoroughly rinsed with potable water prior to reuse.

NOTE: The above directions for use must appear on labeling of products containing cresylic acid, synthetic phenols, pine oils or quaternary ammonium compounds.

SANITIZER TEST
(for inanimate, non-food contact surfaces)

To substantiate the sanitizing claims for a product, the applicant must submit data prior to registration to show that the product, when used as directed, will substantially reduce the numbers of test microorganisms on a surface over that afforded on an untreated control surface. The following protocol may be utilized:

Product samples representing three different preparations must be tested against each test bacterium on each representative surface. In addition, one sample preparation representing a 60-day shelf-life stability study must be tested in duplicate using one test bacterium. The test microorganisms may be: Staphylococcus aureus ATCC 6538 and Klebsiella pneumoniae, aberrant, ATCC 4352; Enterobacter aerogenes ATCC 13048 may be substituted for K. pneumoniae. Representative test surfaces may include, but are not limited to, glass, metal, unglazed or glazed ceramic tile, or vitreous china, depending on the uses proposed on the label. The propagation of cultures and the use of subculture media and other related equipment should be specified in sections 4.001 and 4.002 of the current A.O.A.C. Manual of Methods.

Determine the count of bacteria in an 18 to 24-hour culture and add a 0.01 to 0.03 ml. quantity of the broth culture by spreading on 1 X 1 inch squares of test surface using a bacteriological loop. The squares should be dried for 20 to 30 minutes in a bacteriological incubator at 30 to 37 C. A "zero time" bacterial numbers recovery test must be performed to show the efficiency of the recovery process and must be reported. The n zero time" test should show the loss in viability that occurred during the drying. Apply the product to the test surface as directed on the label. After a suitable time interval, recover test organisms by washing the squares with adequate agitation in appropriate media or diluting fluid containing appropriate neutralizers. Make plate counts on appropriate nutrient agar containing the same neutralizers by the pour or spread plate technique. Exposure time intervals between zero time and ten minutes should be tested for the product as well as for the untreated controls.

The results must show a bacterial reduction of at least 99.9% over the parallel control count within 5 minutes. Three test surfaces with each test microorganism must be tested against each sample for a total of nine determinations with each bacterium. The 60-day shelf-life study should be carried out with two test surfaces against one test bacterium.

BACTERIOSTATS, SANITIZERS AND DISINFECTANTS
USED FOR LAUNDERING TEXTILES

Products Bearing Residual Bacteriostatic Claims on Fabric:

( ) All tests are to be carried out by the A.O.A.C. Bacteriostatic Activity of Laundry Additives Test, as specified in the second Supplement to the 11th Edition, Official Methods of Analysis A.O.A.c. (J.A.O.A.C., 55 (2): 400-402, 1972). Three samples representing 3 product preparations are tested, with five l-inch square treated swatches against S. aureus and Klebsiella pneumoniae, aberrant, ATCC 4352 on seeded agar for each sample. Duplicate samples of one preparation representing 60-day shelf-life stability must be tested with 5 replicate fabric swatches on agar seeded with S. aureus.

Products Bearing Sanitizing or Disinfecting Claims for Fabric and/or Laundry Wash-Water:

An Official test method is not available for assessing the efficacy of products in this category. Refer to the J.A.O.A.C., 52 836-838, 1969 for appropriate test protocols of a tentative method. The gram-negative test bacterium is Klebsiella pneumoniae, aberrant, ATCC 4352. Tests may be carried out in a washing machine or in the devices specified in the tentative method. General protocols, propagation of cultures, fabric to water ratios, test materials, water temperatures and subculture media must be the same as that specified in the tentative method.

( ) Sanitizing Claims: Three samples representing 3 product preparations, each sample tested with 3 replicate fabric swatches against the 2 test bacteria specified. One sample representing a 60-day shelf-life storage must be tested in duplicate against Staphylococcus aureus. A 99.9% reduction within 10 minutes must be demonstrated over the control counts.

( ) Disinfecting Claims: Three samples representing 3 product preparations, each sample tested with 30 replicate fabric swatches against the 2 test bacteria specified. One sample representing 60-day shelf-life storage must be tested in duplicate with 15 swatches against S. aureus. The method must be modified to include testing of both the fabric and the wash water (5 ml from the automatic washer or 0.5 ml from the container with the test device) in individual wide-mouth jars containing subculture media and neutralizers. The added wash water to media volume ratio should not exceed 1:40. Growth or no growth is noted after 48 hours incubation.

The data requirements outlined herein do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones or trichloro-s-triazinetrione.

DUPLICATED PRODUCT FORMULATIONS
Based on Data Provided by Basic Manufacturers

With products proposed for registration that duplicate a formulation which is registered but not intended to be marketed by a basic manufacturer, the data developed and on file may be applied provided:

( ) 1. A formal letter of authorization from the basic manufacturer granting access to his file is submitted.

2. The following efficacy data are developed on the applicant's own finished product:

( ) A. General Disinfectants

(1) A.O.A.C. Use-Dilution Method
(2) Thirty replicates on each of two samples against Salmonella choleraesuis

( ) B. Sanitizers as terminal rinses for glassware, utensils, or equipment in restaurants, bars, dairies and food processing plants.

(1) A.O.A.C. Germicidal and Detergent Sanitizers Test
(2) One test on one sample employing escherichia coli. Test must be performed in proper hard-water if label claim for hard-water tolerance is made.

( ) C. Pressurized spray disinfectants

(1) Certification that all materials and devices making up the container are identical to those specified by the basic manufacturer
(2) A.O.A.CI Germicidal Spray Products Test
(3) Thirty replicates on each of two samples against S. aureus and S. choleraesuis.

PRODUCT FORMULATIONS IDENTICAL
TO REGISTERED PRODUCTS

Products proposed for registration that are identical to products already registered, manufactured and marketed by a basic registrant may be accepted provided:

( ) 1. A formal letter of authorization granting access to the registered product file is submitted.

( ) 2. A document substantiating that the product is manufactured and/or formulated by the basic registrant for the proposed applicant is submitted.

( ) 3. If the proposed product, other than a pressurized spray, is to be manufactured and/or formulated by the applicant, the criteria set forth and marked in enclosure Duplicated Products Formulations must be complied with.

( ) 4. If the product is a pressurized spray, it must be shown that all materials and devices are identical to those utilized by the basic registrant; furthermore, the filler packaging company must be the same for both products. If this cannot be documented and substantiated, the applicant must submit complete efficacy data by the A.O.A.C. Germicidal Spray Products Test on his own product.

FORMULATIONS PRODUCED BY SIMPLE AQUEOUS
DILUTIONS OF REGISTERED CONCENTRATES

The basic efficacy data developed by the supplier of the registered concentrate may be utilized under the following guidelines:

I. Written authorization for use of his file on behalf of the applicant must be submitted by the supplier.

II. Data to confirm that the diluted product can be expected to be effective when used as directed must be developed as follows:

A. Emulsion-based concentrates (phenolics, iodophors, orthodichlorobenzene)

DATA REQUIREMENTS

( ) 1. For "General Disinfectants"

Three samples tested by the A.O.A.C. Use - Dilution Test against Salmonella choleraesuis utilizing 30 replicates per sample.

2. For Sanitizers or Detergent Sanitizers:

Three samples tested with or without hard water (depending on label claims) by the A.O.A.C. Germicidal Detergent Sanitizers Test, three determinations.

B. Pure chemicals and simple formulations (quaternary compounds with or without alcohol, hypochlorites, chlorinate isocyanurates, true pine oil disinfectants)

( ) 1. Control data derived from the first three production batches of the product based on analytical chemical and/or physical methods submitted in response to PR Notice 70-12. This data must be submitted within six months after receipt of this enclosure.

( ) 2. With quaternary-based products bearing disinfecting or hard water sanitizing claims, an analysis of the major impurities found in municipal or other water supply sources used to dilute the product must be provided. A report from the local water authority may serve to satisfy this requirement.

TENTATIVE TEST METHOD FOR LABORATORY EVALUATION OF RUG AND CARPET SANITIZERS OX BACTERIOSTATS

(Revised 4-12-71)

EQUIPMENT

1. Carpet Mounting Board - Mount a piece of 1/8 inch tempered hardboard, with tempered surface up, on a 16 x 16 inch base of 3/4 inch thick marine plywood, with 3/4 inch brads.

2. Cutting Equipment - 2 x 2 inch squares of 1/4 inch acrylic plastic as templates with 3/32 inch holes in the center and a sharp knife (1) (Beaver Office Knife with #27 or #28 replaceable blade).

3. Scrub Brushes - 1-1/4 x 3-1/2 inch surgical hand brush with 5/8 inch nylon bristles (Tomac 29721-010 (2) or equivalent $13.00 per dozen).

4. Extraction Bottles - 8 ounce, widemouth, round polypropylene bottles with screw caps (Naglene 2105 or equivalent) containing 10 stainless steel penicillin cylinders and 100 ml of appropriate neutralizer broth. (Similar style glass bottles may be used, but care to prevent breaking during shaking must be observed). For phenolic based carpet sanitizers, use AOAC nutrient broth with double strength Letheen neutralizer (AOAC Letheen Broth plus an additional 0.7 g. Lecithin and 5.0 gram Tween 80 per liter).

5. Spray Device - Adjustable spray atomizer modified to feed from a calibrated test tube or bottle. (A Model 15, DeVilbiss atomizer on a 2 ounce bottle graduated with 10 mls marks).

6. Carpet - A standard type of nylon, acrylonitrile or wool pile carpeting with a fiber warp-backing. (A velvet nylon carpet with 1/4 inch deep pile on a double jute backing, Perview Pattern, Wanda Weeve Carpet was selected for collaborative testing).

    1) Rudolph Beaver Company, Belmont, Massachusetts
    2) American Hospital Supply, Evanston, Illinois

TEST CULTURES AND MEDIA

1. Test Bacteria - *Staphylococcus aureus* ATCC 6538 and *Enterobacter aerogenes* ATCC 13048.

2. Double Strength Neutralizer Broth - AOAC Letheen Broth with an additional 0.7 g Lecithin and 5.O grams 0Tween 80 per liter or 0.10% sodium thioglycollate and 0.018 isooctylphenoxy polyethoxyethanol in pH 7.2 phosphate buffer.

3. Neutralizer Plant Count Agar - Standard Methods agar (Tryptone Glucose Extract Agar) to which is added 0.7 Lecithin and 5.0 grams Tween 80 per liter.

BACTERIAL INOCULUM

Prepare 8 ounce French square culture bottles with NUTRIENT AGAR B (Methods AOAC*, 4.023 (a), (2)) composed of 3 grams beef extract, 5 grams Anatone and 30 grams agar per liter. Prepare culture suspensions as per section 4.026 (Methods AOAC*). Dilute the standardized suspension in phosphate buffer dilution water (Methods AOAC*, 4.023 (F)) to obtain a stock inoculum suspension containing 10 x 10 organisms per ml.

PROCEDURE

1. Cut the carpet into 8 x 12 inch pieces and with aid of 2 x 2 inch template cut 2 rows of 3 squares per row from the backing side of the carpet having at least 4 inches between the center of each square. The test squares may be completely cut free or a preferred method is to leave about 1/8 of an inch of backing intact at each corner of each cut square so that the entire piece of carpeting can be sterilized and inoculated without separation. A mark is made with a water-proof marking pen (Carter's MARKS-A-LOT or equivalent) in the center of each test square to aid in inoculating the pile surfaces. Cover surface of carpeting with aluminum foil and fold over edges to secure. Steam sterilize and dry. Foil will serve to prevent contamination of controls during spraying. Only carpet that has been determined to be free of residual antimicrobials on the pile or backing surfaces following autoclaving by a zone of inhibition overlay technique should be used.

2. Prepare a standardized stock bacterial suspension by washing the 24 hour growth from NUTRIENT AGAR B bottles and adjust to a density of 10 x 109 organisms per ml with phosphate buffer. Dilute the stock suspension with phosphate buffer containing 0.01% isooctylphenoxy polyethoxyethanol to a concentration of 10 x 10 organisms per ml. Inoculate the previously marked center of each square with 0.1 ml of bacterial suspension. (Retain bacterial suspension for determination of inoculation numbers). Dry inoculated carpet in a warm incubator at 36°C for 60 minutes with the foil wrap losely in place.

   NOTE: The scrubbing procedure should be conducted in a biological hood or in a glove box. A simple safety chamber can be constructed from a large plastic bag.

* OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS' 11th Edition, 1970.

3. Condition brushes by immersing the bristles in separate containers (150 mm glass petri dishes or equivalent) or diluted test solution and a control solution without the active agent for 15 minutes. (If a control solution is not available, use sterile distilled water containing 0.01% isooctylphenoxypolyethoxyethanol). Fasten two pieces of inoculated carpet containing 12 test squares onto a mounting board by nailing each corner with upholsterer's tacks with the foil wrapping positioned so as to protect the controls during the scrubbing treatment. Place board in a safety hood.

4. Apply the portion of the predetermined amount of test solution at a temperature of 25°C uniformly to one piece of carpeting containing six spots of dried bacterial inoculum. (Liquids should be applied by a metered spray based on the amount of solution in excess of 25 ml applied by brushing based on recommended application rate per surface area. Solids may be applied by dusting). Shake excess test solution from conditioned brush and transfer to a dish containing

100 mls of test solution. Dip bristles of brush and transfer amount of retained test solution to a spot on the carpet. Scrub the spot for 30 seconds using 30 circular clockwise strokes and 30 circular counterclockwise strokes. A circular area of pile approximately 3 inches in diameter around each spot should be covered by this treatment. Moderate to heavy pressure should be applied downward on the brush to work the solution to the base of the pile. Repeat dipping of brush into test solution and scrubbing procedure until each of the six spots is treated. Allow the treated carpet to remain at room temperature for 60 minutes for partial drying of treated areas.

5. While the carpet treated with test solution is drying, spray on an amount of non-active control solution equivalent to the surface area onto 3 of the spots of dried inoculum on the control carpet. The remaining 3 spots are unscrubbed controls to determine reduction in inoculation numbers due to drying. Care should be taken not to wet or scrub over the unscrubbed control area. Scrub the 3 wet spots in the same manner as test carpet. Allow the scrubbed controls to dry 60 minutes at room conditions as for test pieces.

6. Following the 60 minute reaction period, cut each 2 x 2 test piece free with flamed forceps and knife. Transfer each 2 x 2 piece of carpeting to a separate extraction bottle of neutralizer broth. Shake each extraction bottle vigorously for a minimum of one minute to free viable organisms from the carpet fibers. Determine the number of viable organisms in each sample bottle by plating duplicate dilutions in Standard Methods Agar with appropriate neutralizer. Determine the number of viable organisms in 0.1 ml of the bacterial suspension used for inoculating the carpet. Incubate all broth extraction bottles to determine germicidal or incomplete neutralization of the test carpet.

7. Determine the percent reduction of viable organisms by the treatment method by comparing the number of surviving organisms of the treated sample against the average viable count of the scrubbed control sample. A count of 1.0 x 106 organisms per ml from the extracted unscrubbed control spots is necessary for a valid test.

If you intend to employ this test to derive data to support the registration of a carpet sanitizer, the following protocols must be considered:

1. Three (3) product samples representing three separate preparations must be tested against each of the two bacteria specified in the method with two different types of carpet. At 99.9% reduction of test bacteria over the control count must be demonstrated in each case. If the product is intended for use on commercial-grade carpeting, two representative carpets such as "Acrylic" and polypropylene tufted-loop type may be tested. No carpeting is available to serve as a standard. If the product is intended for use on wool carpeting, an additional representative sample must be tested. If not, the label must bear a disclaimer for such use. All carpet samples tested must be fully identified, and the pile fiber type, pile yarn weight of finished carpet, pile density and tuft height reported. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the test results.

2. One (1) sample preparation representing a 60-day shelf-life stability study must be tested with one (1) carpet sample and one (1) test bacterium.

The amount of sanitizer applied to a piece of carpet must be extrapolated to gallons of diluted shampoo to square feet of carpet and this must be declared on the proposed label. We would expect this to be 12 gallons of dilute product to 800 to 1200 square feet of carpet. If the application is intended for hospitals, a wet vacuum pickup

must be specified in the label directions. In no case will the so-called dry shampoo treatment be considered for hospital use.

Technical questions concerning the method may be addressed to: Dr. D.F. Garvin, BASF Wyandotte Corporation, Wyandotte, Michigan 48192. Dr. Garvin may also be reached by phone: 313-282-3300.

SPECIAL DIRECTIONS FOR CRESYLIC ACID AND SYNTHETIC PHENOLS

(For Use as Farm Premise Disinfectants)

1. Do not use in milking stalls, milking parlors or milk houses.

2. Remove all animals and feeds from premises, cars, boats, trucks, and other equipment.

3. Remove all litter and manure from floors, walls, and surfaces of barns, pens, stalls, chutes, and other facilities and fixtures occupied or traversed by animals.

4. Empty all troughs, racks, and other feeding and watering appliances.

5. Saturate all surfaces with accepted disinfecting solution.

6. Immerse all halters, ropes and other types of equipment used in handling and restraining animals as well as forks, shovels, and scrapers.

7. Ventilate buildings, cars, boats, and other closed spaces. Do not house livestock or employ equipment until treatment has been absorbed, set or dried.

8. All treated feed racks, mangers, troughs, automatic feeders, fountains and waterers must be thoroughly scrubbed with detergents and rinsed with potable water prior to reuse.

Efficacy Data Requirements for Sterilizing Claims

Employ the Association of Official Analytical Chemists' Sporicidal Test (A.O.A.C. Official Methods of Analysis, 11th Edition, Chapter 4, pp. 64-65, 1970) using:

(a) Three samples representing three different preparations using both types of carriers (porcelain penicylinders and surgical silk suture loops) and both test organisms (Bacillus subtilis and Clostridium sporogenes). Thirty replicates are required with each organism on each type of carrier. (Total of 120 replicates or carriers for each sample).

(b) Duplicate samples of one preparation representing a 60-day shelf-life stability study using 30 replicates with both test organisms named above on both types of carriers. (Total of 120 replicates or carriers for each sample).

The total number of tubes required in a single test using (a) and (b) above is 600. For the sterilizing claim, no failure in any replicate is permitted.

Methods for Evaluating the Virucidal Activity of Disinfectants

No specific official standardized method for evaluation the virucidal activity of disinfectants has been established at the present time. Until an official method has been adopted, the Division will accept adequate data developed by any virological technique which is recognized as technically sound. The procedure employed must stimulate actual in-use conditions. For this reason, acceptable virological data are usually developed by carrier methods which are modifications of either the A.O.A.C. Use-Dilution Method (for liquid surface disinfectants), or the A.O.A.C. Germicidal Spray Test (for spray surface disinfectants). In order to simulate in-use conditions, the specific virus to be tested 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. The treated surfaces must be assayed after ten-minutes exposure to determine if the virus has been inactivated. Since most germicidal chemicals are inherently toxic for the host systems employed in virological techniques (especially cell cultures), either a "detoxification" procedure which will render the treated virus free of residual germicide, or a virus concentration of sufficient titer to demonstrate at least a three-log reduction even when cytotoxicity is observed in tissue culture systems must be employed in the study.

The following information must be provided on each test:

1. The dilutions employed in the recovery of the virus film from the surface; e.g. 10-1, 10-2, etc.

2. Virus controls: Surfaces contaminated with the test virus; no germicide used; virus recovered at various dilutions in assay system (tissue culture, embryonated egg, mouse injection, etc.).

3. The activity of the germicide on a minimum of five separate surfaces (e.g. glass slides) contaminated with the test virus, showing recovery at various dilutions in assay system.

4. Cytotoxicity controls: Germicide on surfaces without test virus; effect on assay system.

5. The I.D.50 values calculated in the various tests.

6. The reduction of the virus titer by the activity of the germicide I.D. /ml of the virus control less the $I.D._{50}$/ml of the test system; expressed as Log_{10} and calculated by a statistical method Reed and Muench: Litchfield and Wilcoxan, as examples).

A typical laboratory report involving a tissue culture, therefore, would include the details of the methods employed together with a table such as follows:

Dilution of Virus from Surface	Virus-Germicide (Recovery* of Virus Treated Surface)	Virus-Control (Recovery* of from Virus from Untreated Surface)	Cytotoxicity-Control (Toxic Effect of Germicide; No Virus)
10^{-1}	T T T T T	+ + + + +	T T T T T
10^{-2}	T T T T T	+ + + + +	T T T T T
10^{-3}	T T T T T	+ + + + +	T T T T T
10^{-4}	T T T T T	+ + + + +	T T T T T
10^{-5}	T T T 0 0	+ + + + +	T T T T T
10^{-6}	0 0 0 0 0	+ + + + +	O O O O O
10^{-7}	0 0 0 0 0	+ + + + +	O O O O O
10^{-8}	0 0 0 0 0	+ + + + +	O O O O O
10^{-9}	0 0 0 0 0	+ O O O O	O O O O O

*Demonstrated by cytopathogenic effect, flourescent antibody, plaque count, animal response, or other recognized acceptable technique

Note: T - Toxic, + - Virus recovered, 0 - No virus recovered

Claims of virucidal activity for a product must be restricted to those viruses which have actually been tested. Duplicate studies on one sample are required for each virus tested. When cytotoxicity is evident (as in above table) at least a 3-log reduction in titer must be demonstrated. The virucide must demonstrate complete inactivation of the virus at all dilutions.

Where the test virus does not demonstrate measurable cytopathogenic reactions in subculture growth systems, in vitro testing would be considered presumptive. In this case, confirmatory testing in vivo must be carried out in a susceptible host after exposure virus to the disinfectant.

TENTATIVE RESIDUAL (SELF-SANITIZING) METHOD

To substantiate residual self-sanitizing claims for a product, the applicant must submit data prior to registration to show that the product when used as directed will substantially reduce the numbers of test microorganisms on treated representative surfaces; when compared to the numbers of microorganisms on control surfaces which have been treated with the same product without the active ingredient incorporated. The following protocol may be utilized:

Three product samples representing three different preparations must be tested against each test bacterium on at least five replicates of each representative surface for which the product is intended for use. In addition, one sample preparation representing a 60-day shelf-life stability study must be tested in duplicate using one test bacterium on at least five replicates of each representative surface to be treated. The test microorganisms may be: Staphylococcus aureus ATCC 6538 and Klebsiella pneumoniae, aberrant, ATCC 4352. Enterobacter aerogenes ATCC 13048 may be substituted for K. pneumoniae. Representative test surfaces may include, but are not limited to glass, metal, unglazed or glazed ceramic tile, vistreous china, or wooden flooring depending on the uses proposed on the labels. The propagation of cultures and the use of subculture media and other related equipment should be as specified in sections 4.001 and 4.002 of the current A.O.A.C. Manual of Methods.

Apply the product to the control and test surfaces as directed on the label, allowing for complete drying on the surfaces. Determine the count of bacteria in a 48-hour broth culture and add a 0.01 to 0.03 ml quantity of the broth culture by spreading on 1 X 1 inch squares of each test surface using a bacteriological loop. The squares should be dried for 20 to 30 minutes in a bacteriological incubator at 30 to 37 C. A "zero time" bacterial numbers recovery test must be performed to show the efficiency of the recovery process and must be reported. The "zero time" test should show the loss in viability that occurred during the drying. A minimum count of 1 X 106 organisms per test surface must be recovered after drying for the test to be considered valid.

After a suitable time interval recover test organisms by washing the squares with adequate agitation in appropriate media or diluting fluid containing a the same neutralizers by the pour or spread plate technique. Exposure time intervals between zero time, thirty minutes, one tour, three hours and six hours should be tested and reported for each surface treated with the product, as well as for each of the controls.

The results must show a bacterial reduction of at least 99.9% over the parallel control count within three hours to demonstrate any practical value.

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