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TECHNICAL SUPPORT SECTION EFFICACY REVIEW - I

Disinfectants Branch

IN 05-13-86 OUT 07-08-86

Dennis G. Guse WEC 7/9/86

Reviewed By Dennis G. Guse Date 07-08-86

EPA Reg. No. or File Symbol 8383-3

EPA Petition or EUP No. None

Date Division Received 04-28-86

Type Product Hospital/General Disinfectant

Data Accession No(s). 262749 MRID 159556

Product Manager 32 (Kempter)

Product Name Permacide Brand (Ristex) Germicidal Disinfectant

Company Name Sporicidin International

Submission Purpose Amendment to add virucidal (AIDS/HTLV-III, Herpes simplex Types 1 and 2, Polio Type 1) and tuberculocidal claims with data, label, and bulletin

Type Formulation Ready-to-use liquid

Active Ingredient(s):	%
Phenol	1.41
Sodium phenate	0.24
Sodium borate	0.47

200.0 Introduction

200.1 Uses

The product is registered (last accepted label 02-02-73) as a hospital/general disinfectant (Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella choleraesuis, Group A alpha hemolytic Streptococcus, Group A beta hemolytic Streptococcus, Streptococcus viridans, Escherichia coli), pathogenic fungicide (Trichophyton interdigitale). and virucide (Influenza A) when used full strength on previously cleaned (when necessary) surfaces and articles, including thermometers, instruments, telephones, tubing, and other disposable and reusable supplies and equipment (natural and synthetic hard surfaces made from plastics, latex, glass, finished wood, metal, porcelain, enamel, and painted surfaces) in hospitals, institutions, and households.

It is also recommended to kill odor producing organisms, and mold and mildew. It bears fungicidal claims against Aspergillus niger (mold and mildew), Chaetomium globosum (paper and textile spoilage), and Pellicularia filimentosa (paint spoilage).

It also bears claims as a deodorizer and self-sanitizer for many uses.

200.2 Submission Contents

The current submission consists of additional data intended to support additional virucidal (AIDS/HTLV-III, Herpes simplex Types 1 and 2, Polio Type 1) and tuberculocidal claims. A revised label and technical bulletin were also submitted.

200.3 Factors affecting Review

Since the proposed amendment includes significant additional human health related efficacy claims, review of previous and current efficacy data submitted for this product, as well as the proposed labeling, is required in the light of current, updated requirements.

201.0 Data Summary

201.1 Previously Submitted Data

201.1.1 Brief Description of Tests

Data submitted for EPA Reg. No. 8383-3 under Accession Nos. 006136, 006138, 006139, 018092, 103620, 103627, and 221375, dated 02-03-70 to 01-03-73.

201.1.2 Test Summaries

Efficacy data developed by the AOAC Use-Dilution Method was accepted to support product effectiveness on precleaned, hard, non-porous surfaces at 10 minutes contact time against Staphylococcus aureus, Salmonella choleraesuis, Pseudomonas aeruginosa, Trichophyton interdigitale, Escherichia coli, Streptococcus salivarius (alpha hemolytic streptococci), and Streptococcus pyogenes (beta hemolytic streptococci) (Reported by Dr. Eddie D. Leach, Milligan College, Tennessee and Dr. Martha C. Sager, American University, Washington, D.C.). (2)

Also included were electron photomicrographs of untreated and treated cells of Staphylococcus aureus showing cytological effects (dehydrated cytoplasm, altered chromatin mass, and destroyed cell wall and membrane).

Efficacy data developed by virucidal tests with liquid virus suspensions was accepted to support product effectiveness against Influenza A virus (Influenza A/PR/8/34). These data are not acceptable in meeting current requirements for virucidal testing with virus dried on a hard surface carrier.

Efficacy data were also developed to support fungicidal, fungistatic, and bacteriostatic claims against non-human health related organisms, including Penicillium variabile, Aspergillus niger, Chaetomium globosum, and Pellicularia filimentosa. These data are subject to the efficacy data waiver and are no longer required to be submitted.

201.2 Currently Submitted Data

201.2.1 Brief Description of Tests (Accession No. 262749)

a. "AOAC Tuberculocidal Efficacy Test with 5% Added Soil Using Permicide Concentrate in a Spray Bottle Delivery System" report by Dr. Kyle H. Sibinovic, Shaldra Biotest, Inc., Bethesda, MD 20817, dated 02-15-86

b. "Permacide Brand (Ristex) Germicidal Disinfectant 10/7/85 vs. Herpes Simplex Virus-1 (F-Strain), Herpes Simplex Virus-2 (G-Strain), and Poliovirus-1 (Brunhilde-ATCC VR-58)" reports by Dr. Philip R. Roane, Integrity Biosrvices, Inc., Rockville, MD 20852, dated 12/12/85.

c. Permacide Inactivation of HTLV-III Virus. Report by Sue C. Tondreau, Bionetics Research, Inc., Kensington, MD 20895-1078, dated 01-07-86.

201.2.2 Test Summaries

a. AOAC Tuberculocidal Activity Method (II. Confirmative In Vitro Test)

1. Modifications: 5% horse serum added as organic soil.

2. Samples: "Permacide Concentrate", Lot A (K0253) and Lot B (J1453).

3. Dilution: Undiluted.

4. Exposure: 10 minutes at 20°C.

5. Test Organism: Mycobacterium bovis (BCG). Phenol sensitivity 1/50 and phenol resistance 1/70 in 10 minutes at 20°C.

6. Subculture medium/Neutralizer: Modified Proskauer-Beck broth (MPB), 7H9 broth (7H9), and Kirchner's broth (KIR) employed as subculture media, and letheen broth used as neutralizer.

7. Incubation: 60 days at 37°C; negative or very faint growth re-incubated for an additional 30 days.

8. Results:

Test Sample & Lot No.	Positive/Total Carriers in Subculture Medium		
	MPB	7H9	KIR
Permacide Lot A	0/10	0/10	0/10
Permacide Lot B	0/10	0/10	0/10

9. Conclusions: Acceptable performance of product as a tuberculocide used undiluted for 10 minutes at 20°C.

b. Virucidal Tests vs. Herpes simplex Types 1 and 2 and Poliovirus Type 1

The submitted test reports are incomplete and cannot be evaluated because the procedures used in developing the virucidal data were not included with the test results.

In addition, test results were included for only 1 sample of the product with each virus instead of 2 samples of product, representing 2 different batches, with each virus as required.

c. Virucidal Tests vs. HTLV-III

1. Procedures: Two procedures were employed, as follows -

A. Method 1 (Part A) By Simple Dilution: 0.01 ml of (10,000X) virus inoculum was placed in a sterile tube (Groups I, II, III, and IV). The inoculated surface area was unspecified. The virus was air-dried under unspecified time and temperature conditions. The virus was then suspended in 0.1 ml of disinfectant (Groups I, II, and III) or media (Group IV). After 1 minute (Group I), 5 minutes (Group II), or 10 minutes (Group III) at 20°C, the virus-disinfectant mixtures were diluted with 10 ml (1/100) and 1000 ml (1/10,000) of 1/100 disinfectant. Control (Group IV) was diluted with 100 ml to 1,000,000 ml of a 1/100 dilution of disinfectant. Then all sample dilutions were assayed for cell infectivity determinations. The virus-disinfectant mixtures (Groups I, II, and III) were assayed at 1/100 and 1/10,000 dilutions. The virus control (Group IV) was assayed at 1/1,000 to 1/10,000,000 dilutions. No cytotoxicity control was included.

B. Method 2 (Part B) By Pelleting To Recover Virus: 0.01 ml of (10,000X) virus inoculum (without drying) was incubated with 0.09 ml of disinfectant. Since the virus inoculum was not dried on a surface, as required, this method is unacceptable and was not evaluated.

2. Modifications: None reported.

3. Sample: "Permacide". Only 1 sample tested.

4. Dilution: The tables of results refer to "1:16 Permacide". However, the balance of the report refers to "undiluted Permacide".

5. Exposure: Nominally 1, 5, and 10 minutes at 20°C with undiluted disinfectant. However, all samples were diluted and assayed in a 1/100 dilution of disinfectant.

6. Test Virus: HTLV-III. The test strain was not identified.

7. Host Cells and Assay System: The host cells and media employed were not identified. For infectivity determinations, 1 ml of sample was incubated with 1 x 10^7 cells for 90 minutes at 37°C for virus adsorption. Following adsorption, cells were washed once, resuspended in 20 ml of media, and distributed into quadruplicate tissue culture flasks. Cultures were incubated for up to 25 days and monitored for reverse transcriptase (RT) activity (dT.rA). Counts per minute (CPM) greater than 10,000 were considered positive for virus.

8. Results: See next page for results by Method 1 (Part A).

9. Conclusions: Performance of the product as a virucide vs. HTLV-III on inanimate surfaces could not be evaluated because -

   A. Method 1 (Part A) By Simple Dilution:

      i. The procedure for drying the virus on a surface was inadequate.

      ii. The technique of resuspending the virus in the disinfectant was unacceptable.

      iii. The effect of the disinfectant exposure could not be determined since the diluent employed was inappropriate and the controls were inadequate.

      iv. Only 1 sample of disinfectant was tested instead of 2 samples, representing 2 different batches, as required.

      v. The test report was deficient.

   B. Method 2 (Part B) By Pelleting To Recover Virus:

      The procedure employed and results obtained were not evaluated since the virus was not dried on a surface as required.

Method 1 (Part A)

Virus Dil.	Exposure (Dis. Dil.)	Exposure (Min. @ 20°C)	Diluent (Dis. Dil.)	Virus (+), No Virus/Not Toxic (o), or Toxic (T) After 25 Days: Virus-Disinfectant	Virus Control	Cytotoxicity Control
10^{0}				----	----	----
10^{-1}				----	----	----
10^{-2}	10^{0}	1	10^{-2}	oooo	----	----
	10^{0}	5	10^{-2}	oooo	----	----
	10^{0}	10	10^{-2}	oooo	----	----
10^{-3}	None	10	10^{-2}	----	oooo	----
10^{-4}	10^{0}	1	10^{-2}	oooo	----	----
	10^{0}	5	10^{-2}	oooo	----	----
	10^{0}	10	10^{-2}	oooo	----	----
	None	10	10^{-2}	----	oooo	----
10^{-5}	None	10	10^{-2}	----	oooo	----
10^{-6}	None	10	10^{-2}	----	oooo	----
10^{-7}	None	10	10^{-2}	----	oooo	----

---- = Not Done
oooo = Negative RT; CPM Less Than 10,000/Apparently No Cytotoxicity

TECHNICAL SUPPORT SECTION EFFICACY REVIEW - II

Disinfectants Branch

EPA Reg. No. or File Symbol 8383-3

Date Division Received 04-28-86

Data Accession No(s). 262749

Product Manager No. 32 (Kempter)

Product Name Permacide Brand (Ristex) Germicidal Disinfectant

Company Name Sporicidin International

202.0 Recommendations

202.1 Efficacy Data Supporting Human Health-Related Claims

a. Previously submitted data on file for this product are adequate to support effectiveness as a hospital/general disinfectant/fungicide vs. infectious microorganisms on pre-cleaned, hard, non-porous surfaces that are thoroughly wet by the undiluted solution for a contact time of 10 minutes.

The above data support specific efficacy claims against the following human pathogens:

Staphylococcus aureus
Salmonella choleraesuis
Pseudomonas aeruginosa
Escherichia coli
Streptococcus salivarius ("viridans"/alpha hemolytic streptococci)
Streptococcus pyogenes (Group A beta hemolytic streptococci)
Trichophyton interdigitale/mentagrophytes (athlete's foot fungi)

b. Currently submitted data for this product by the AOAC Tuberculocidal Actvity Method are acceptable to support effectiveness as a tuberculocide vs. Mycobacterium tuberculosis (tubercle bacillus) under the same conditions indicated above.

202.2 Human Health-Related Claims Not Supported by Efficacy Data

a. Previously submitted virucidal data against Influenza A (PR/8/34) virus are no longer adequate in meeting current requirements since the tests were done with liquid virus suspensions, not with the virus dried on a hard surface carrier as currently required.

There is no data to support a claim against Influenza A2 (Hong Kong) virus.

There is no data to support a self-sanitizing claim.

There is no data to support a claim as a disinfectant on porous surfaces.

b. Currently submitted virucidal test results against Herpes simplex Types 1 and 2 and Poliovirus Type 1 were incomplete and cannot be evaluated. The procedures employed in developing the data were not provided. In addition, test results were included for only 1 sample of the product with each virus instead of 2 samples of the product, representing 2 different batches, as required.

c. Currently submitted virucidal data against AIDS/HTLV-III virus are inadequate and unacceptable. The data were deficient with respect to the following:

1. Method 1 (Part A)

(7)

A. The procedure for drying the virus inoculum in the bottom of a tube under uncontrolled/unspecified conditions was inadequate and unacceptable.

B. The technique to "suspend" the virus inoculum with the disinfectant was not acceptable.

C. The effect of virus exposure to the disinfectant could not be assessed since the diluent employed (a 1/100 dilution of disinfectant) was apparently also virucidal and, therefore, unsuitable and uncontrolled.

D. The amount of product testing was insufficient since only 1 sample was tested instead of 2 samples from 2 different batches as required.

2. Method 2 (Part B)

This method was unacceptable since the testing was done with a virus suspension, not with the virus dried on a hard surface carrier as required.

3. Test Report

A. The strain of HTLV-III employed in testing was not identified or described, and no references were provided.

B. The method used in propagating the virus stock and the procedure emplyed to apparently concentrate the virus ("10,000X", "1,000X") were not reported.

C. The titer of the virus inoculum before and after drying was not determined and reported, and the effect of drying on the virus could not be ascertained.

D. References to virus concentration in terms of "X" numbers are vague and confusing. References to disinfectant dilutions which appear in the report are also confusing since it is not clear whether 1:100, etc., refer to the undiluted product (text) or a 1:16 use-dilution (tables).

E. The host cell system employed the specific method used for quantitative assay (reverse transcriptase activity) were not described and no references were provided.

F. Valid and acceptable controls for (positive) virus, cytotoxicity, and the neutralized/diluted disinfectant were not included.

202.3 Additional Data Required to Support Human Health-Related Claims

a. To support a claim against Influenza A2 (Hong Kong) virus, data must be submitted as indicated in the attached DIS/TSS-7 enclosure.

To support a self-sanitizing claim, data must be submitted in accordance with the attached enclosure headed "Residual self-sanitizing activity of dried chemical residues on hard inanimate surfaces".

To support a claim for porous surfaces, all required data to support label efficacy claims must be developed by modified methods employing a porous hard surface carriers as indicated in item 2 of the attached DIS/TSS-2 enclosure.

b. In order to evaluate the submitted virucidal test results against Herpes simplex Types 1 and 2 and Poliovirus Type 1, the complete procedure(s) employed in developing the data must be submitted. In addition, data must be included for 2 samples of the product, representing 2 different batches, against each virus tested.

c. Before any protocol can be considered for determination of the virucidal activity of a disinfectant against HTLV-III/LAV (AIDS) virus on inanimate surfaces, more complete information and specific data must be provided, as follows:

1. Specific identification and description of the strain(s) of AIDS virus employed in testing, including pertinent literature citations, method(s) used in propagating the virus stock, and the titer and composition of the virus inoculum.

2. Specific description of the method employed for quantitative assay of the infective virus (ID-50), including pertinent literature citations, the host cell system used, and the details of the assay procedure.

3. Controlled experimental data showing quantitative survival of the virus(es), as indicated in 202.3(c)(1) and (2) above, on a hard surface carrier before and after drying under specified conditions. These data are necessary to determine the feasibility of any drying procedure in virucidal studies of disinfectants against the AIDS virus on inanimate surfaces which are consistent with the efficacy requirements for EPA registration. Factors to be considered in developing the data are:

A. Volume of virus suspension to be inoculated, and the type and surface area of the carrier employed.

B. Time, temperature, and exposure conditions employed in the drying procedure.

C. Susceptibility of different strains of the AIDS virus to the lethal effects of drying; i.e., two or more strains must be included.

D. Influence, if any, of additives such as serum or plasma, and the effect of virus concentration on survival of the virus dried on surfaces.

4. Based on the deficiencies cited in 202.2(c)(1) and (2) above, and the additional data specified in 202.3(c)(1) to (3) above, the submitted protocol must be revised to minimally include the following:

A. A specific procedure of demonstrated feasibility for drying the virus inoculum on a hard surface carrier.

Usual drying conditions employed in virucidal studies with disinfectants consist of 0.2 ml of virus inoculum spread in a uniform film over the flat bottom surface of a 60-mm petri dish (28 cm^2) air dried for 20 to 60 minutes at 35-37°C.

After adequate drying, a virus control titer of at least 10^4 10^6 must be recovered from the surface.

B. The disinfectant treatment must be applied in a manner so as to cover or immerse, but not "suspend", the dried virus film.

C. Adequate controls must be included to assess the untreated virus and the presence or absence of virucidal and cytotoxic activity of neutralized or "non-toxic" dilutions of disinfectant in the samples.

D. Two samples of disinfectant, representing 2 different batches, must be tested with each virus.

5. Any submitted test report must also be revised to correct the deficiencies cited in 202.2(c)(3)(A) to (F) above.

6. Reprints or copies of referenced articles for any literature citations must be submitted.

7. Refer to the attached DIS/TSS-7 enclosure for guidance in developing and reporting of virucidal test data.

8. It is suggested that any revised protocol be submitted for review prior to the initiation of additional tests.

203.0 Labeling

The following apply to both the product label and the technical brochure, wherever applicable:

a. Retention of virucidal claims is dependent upon the submission of additional, acceptable virucidal data.

b. In lieu of data, claims for self-sanitizing activity must be deleted or qualified as against odor causing bacteria only.

c. In lieu of data, delete claims for disinfection of porous surfaces.

d. The directions for use of the product must include the following instructions:

1. That surfaces must be clean prior to application of the the product.

2. That treated surfaces must remain wet for at least 10 minutes.

e. The broad claim "Continuous Antimicrobial Activity For Over 6 Months" is misleading and unacceptable. "Antimicrobial Activity" is too inclusive a term since all efficacy claims for the product, including all human health-related claims, are included under "antimicrobial activity", whereas the type of claim in question relates only to residual bacteriostatic and fungistatic activity against odor causing bacteria and/or mold and mildew fungi on treated surfaces which are likely to become wet and only as long as the residual chemical is not rinsed or wiped from the surface or contaminated with soil. Under these limitations, the residual activity could not be expected to be "continuous" or to remain for any specific period of time such as "6 months" since this would depend on many factors which are not predictable for all surfaces.

An acceptable claim would be for "Residual Bacteriostatic/Fungistatic Activity", which must be further qualified on the labeling to specify that such activity is limited to "odor causing bacteria and/or mold and mildew fungi in the presence of moisture and when the residual chemical is not removed or inactivated by soil". Therefore, the claims must be revised and qualified accordingly.

f. The claim for reducing "cross infection" is a drug claim. "Cross infection" must be changed to "cross contamination".

g. The claims against disease causing organisms (human pathogens) must be be delineated and separated from claims against odor causing organisms and mold and mildew fungi (not related to human health) wherever they appear on the labeling.

h. Delete "safe".

i. Change "biocide" to "microbiocide".

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Additional label revisions may be required after the efficacy data deficiencies are resolved.

EFFICACY DATA REQUIREMENTS

Supplemental Recommendations

When an antimicrobial Agent is intended for a use pattern that is not reflected by the test conditions specified in the Recommended Methods, one or more test conditions specified in the method must be modified and/or supplementary data developed in order to provide meaningful results relative to the conditions of use. The following basic information is critical to the development and submission of appropriate data.

1. EXPOSURE PERIOD

All products tested by the recommended methods may be tested at the exposure periods prescribed in those methods. However, if the product is intended for use at exposure periods shorter or longer than those specified in the method, the method must be modified, in a manner acceptable to the Agency, to reflect the deviation in exposure intended. A modification to provide a shorter exposure period is restricted by the manipulative limitations inherent in the method, while a modification to provide a longer exposure period is restricted by the conditions applicable to the use pattern. If a ten-minute exposure period is necessary for the antimicrobial agent to be effective against the test microorganism the product cannot be represented as an "instantly active" product, or cannot be represented as being "effective in 30 seconds,""one minute," or at any time period shorter than 10 minutes. Also, the product cannot be recommended for use in a manner which is inconsistent with the exposure period necessary for effectiveness (as, for example, "Spray on surface, and immediately wipe with clean cloth") unless the standard method has been modified and reflects efficacy under such conditions of use. In any case, the exposure period or manner of use necessary to provide efficacy must be featured prominently on the product label.

2. TYPE OF SURFACE

When an antimicrobial agent is intended to be effective in treating a hard porous surface, some of the Recommended Methods may be modified to simulate this more stringent condition by substitution of a porous surface carrier (such as a porcelain penicylinder or unglazed ceramic tile) for the non-porous surface carrier (stainless steel cylinder or glass slide) specified in the method. In addition, control data, described below in Supplemental Recommendation No. 6, must be developed to assure the validity of the test results when this modification of the method is employed. In no case may a surface carrier which represents a less stringent condition be substituted for a surface carrier which is specified in the Recommended Method.

3. HARD WATER

The Recommended Methods may be modified to demonstrate the effectiveness of an antimicrobial agent in hard water. The hard water tolerance level may differ with level of antimicrobial activity claimed. To establish disinfectant efficacy in hard water, all microorganisms (bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

4. ORGANIC SOIL

An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy by the appropriate method(s) which have been modified to include a representative organic soil such as 5% blood serum. A suggested procedure to simulate in-use conditions where the antimicrobial agent is intended to treat dry inanimate surfaces with an organic soil load involves contamination of the appropriate carrier surface with each test microorganism culture containing 5% v/v blood serum (e.g., 19 ml test microorganism culture + 1 ml blood serum) prior to the specified carrier-drying step in the method. Control data, described below in Supplemental Recommendation No. 6, must also be developed to assure the validity of the test results when this modification is incorporated into the method. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. When the surface to be treated has heavy soil deposits, a cleaning step must be recommended prior to application of the antimicrobial agent. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level when specifically claimed and/or indicated by the pattern of use. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test, involves adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5×10^6 /ml) of conidia.

5. RE-USE

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

6. MICROORGANISM SURVIVAL AFTER DRYING ON A HARD SURFACE

Quantitative determinations of the viable microbial concentration on the untreated control carrier after drying are required in order to determine the validity of the test results obtained with treated carriers when the Recommended Methods are modified to include such elements as (i) test microorganisms not specified in the method, (ii) substitution of a porous surface (e.g., porcelain penicylinder, unglazed ceramic tile) for the specified nonporous surface (stainless steel cylinder, glass slide), and/or (iii) an organic soil load. The detailed protocol for this testing must include: (i) preparation of inoculum, (ii) application of inoculum to the carrier, (iii) the time/temperature and relative humidity conditions for drying the microorganisms on the carrier, (iv) the technique for removal of the microorganisms from the carrier, and (v) the specific assay procedure indicating such details as replication, subculture media/diluents, and the incubation time/temperature conditions for the enumeration procedure employed. The test results must include the individual counts obtained by the method.

7. NEUTRALIZATION

For each antimicrobial product, procedures must be employed that will preclude residual effects of the active ingredient(s) in the subculture medium. A specific medium capable of neutralizing the antimicrobial effects of a product (whenever one is known) should be employed prior to the microbiological assay. Some of the Recommended Methods rely solely upon the selection of an appropriate subculture medium to neutralize the antimicrobial effects of certain general types of chemical compounds (active ingredients). However, to document absence of residual effects of the active ingredient(s) in the subculture medium, the following testing is necessary: (i) secondary subcultures must be performed to demonstrate that antimicrobial effects were overcome, or (ii) at the conclusion of the incubation period specified or employed in the method, the primary culture medium with test carrier must be inoculated with approximately 10 microorganisms/ml of the specific culture under test (documented by actual plate counts) and reincubated for the specified period to demonstrate that the subculture medium was capable of supporting bacterial growth.

8. BATCH REPLICATION FOR MODIFIED TESTS

Where the required batch replication has already been performed and accepted for a product registration with unmodified tests by the Recommended Methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carrier, etc.) may be conducted with reduced batch replication, as follows: (i) for basic efficacy claims (e.g., sterilizers, disinfectants, or sanitizers), 2 samples, representing 2 different batches, instead of 3, and (ii) for supplemental efficacy claims (e.g., fungicides, virucides, or tuberculocides), one sample instead of 2.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EFFICACY DATA REQUIREMENTS:

VIRUCIDES

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976)

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. For virucides whose use-directions identify the product as one intended for use upon dry, inanimate, environmental surfaces (such as floors, tables, cleaned and dried medical instruments, etc.), carrier methods, which are modifications of either the AOAC Use-Dilution Method (for liquid surface disinfectants) or the AOAC Germicidal Spray Products Test (for surface spray disinfectants), must be used in the development of the virological data. 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. 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 (petri dish, glass slide, steel cylinder, etc.) for a specified exposure period at room temperature. The virus is then assayed by an appropriate virological technique. The protocol for the viral assay must provide the following information:

(i) The virus recovery from a minimum of 4 determinations per each dilution in the assay system (tissue culture, embroyonated egg, animal infection, or whatever assay system is employed).

(ii) Cytotoxicity controls: The effect of the germicide on the assay system from a minimum of 4 determinations per each dilution.

(iii) The activity of the germicide against the test virus from a minimum of 4 determinations per each dilution in the assay system.

(iv) Any special methods which were used to increase the virus titer and to detoxify the resisdual germicide.

(v) The The ID-50 values calculated for each assay.

(vi) The test results shall be reported as the reduction of the virus titer by the activity of the germicide (ID-50 of the virus control less the ID-50 of the test system), expressed as $\log_{10}$ and calculated by a statistical method (Reed and Muench, 1938; Litchfield and Wilcoxon, 1949; as examples).

(vii) For virucidal data to be acceptable, the product must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident (as in attached tables) at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. The calculated viral titers must be reported with the test results.

A typical laboratory report of a single test with one virus (recovered from a treated surface) involving a tissue culture, therefore, would include the details of the methods employed and the information in the attached tables.

Claims of virucidal activity for a product must be restricted to those viruses which have actually been tested. Separate studies on two batches of product are required for each virus.

References

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. Jour. Pharma. Exp. Therapy, 96: 99-113.

Reed, L. J., and H. Muench. 1938. A simple method of estimating 50 per cent end-points. Amer. Jour. Hygiene, 27: 493-497.

Table 1 - Test Results

Dilution of Virus	Virus - Disinfectant[1]	Virus - Control[1]	Cytotoxicity-Control
10^{-1}	T T T T	+ + + +	T T T T
10^{-2}	T T T T	+ + + +	T T T T
10^{-3}	T 0 0 0	+ + + +	T 0 0 0
10^{-4}	0 0 0 0	+ + + +	0 0 0 0
10^{-5}	0 0 0 0	+ + + +	0 0 0 0
10^{-6}	0 0 0 0	+ + + 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0

[1] Recovery of virus from surfaces demonstrated by cytopathogenic effect, fluorescent antibody, plaque count, animal response, or other recognized acceptable technique.

NOTE: T = toxic; + = virus recovered; 0 = no virus recovered.

Table II-Calculation of the Tissue Culture Infective Dose 50 ($TCID_{50}$)

Virus dilution inoculated	No. infected/ No. inoculated	No. infected	No. not infected	ACCUMULATED VALUES No. infected	No. not infected	No. infected/ No. inoculated	per cent infected
10^{-1}	4/4	4	0	24	0	24/24	100
10^{-2}	4/4	4	0	20	0	20/20	100
10^{-3}	4/4	4	0	16	0	16/16	100
10^{-4}	4/4	4	0	12	0	12/12	100
10^{-5}	4/4	4	0	8	0	8/8	100
10^{-6}	3/4	3	1	4	1	4/5	80
10^{-7}	1/4	1	3	1	4	1/5	20
10^{-8}	0/4	0	4	0	8	0/8	0

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

Table III-Calculations of the Tissue Culture Lethal Dose 50 ($TCLD_{50}$)

Virus dilution inoculated	No. toxic/ No. inoculated	No. toxic	No. not toxic	ACCUMULATED VALUES No. toxic	No. not toxic	No. toxic/ No. inoculated	per cent toxic
10^{-1}	4/4	4	0	9	0	9/9	100
10^{-2}	4/4	4	0	5	0	5/5	100
10^{-3}	1/4	1	3	1	3	1/4	25
10^{-4}	0/4	0	4	0	7	0/7	0
10^{-5}	0/4	0	4	0	11	0/11	0
10^{-6}	0/4	0	4	0	15	0/15	0
10^{-7}	0/4	0	4	0	19	0/19	0
10^{-8}	0/4	0	4	0	23	0/23	0

$TCLD_{50} = 10^{2.7}$

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

Claims for virucidal activity for a product must be restricted to those viruses which have actually been tested.

(m) 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 (i.e., significant reduction in numbers of infectious microorganisms which may be present or subsequently deposited) on treated surfaces that are likely to become and remain wet under normal conditions of use.

(1) Test standard. Each test must include the following basic elements:

(i) It must be based upon an adequately controlled in-use study or simulated in-use study employing as test microorganisms those target pathogens that are likely to be encountered in the environment in which the product is to be used.

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

(iii) The residue on the treated surface(s) 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 type(s) of surface 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, such as relative humidity and temperature, employed in the test must also be reported; these must be the same as those which are likely to be encountered under normal conditions of use.

(5) Performance standard. For residual self-sanitizing claims, it must be demonstrated that at least 99.9% reduction in the numbers of test microorganisms occurred on the treated surface(s) over that of the parallel control surface(s).

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Page_____ is not included in this copy.

Pages 22 through 24 are not included.

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

X A draft product label.

____ The product confidential statement of formula.

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