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# Product Performance Test Guidelines

## OPPTS 810.2100: Sterilants—Efficacy Data Recommendations



Public Review Draft

## NOTICE

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

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

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

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## **OPPTS 810.2100: Sterilants—efficacy data recommendations.**

### **(a) Scope**

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

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

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

### **(c) General considerations**

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

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

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

**(1) Test procedure.** The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method II (Ref. 1) to demonstrate the sterilant efficacy of products. Sixty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) should be tested against spores of both *Bacillus subtilis* (*B. subtilis*) (American Type Culture Collection (ATCC) 19659) and *Clostridium sporogenes* (*C. sporogenes*) (ATCC 3584) on three samples representing three different batches of the product, one of which should be at least 60 days old (240 carriers per sample, or a total of 720 carriers). The inoculum employed should provide a count of  $1 \times 10^5 - 1 \times 10^6$  colony forming units per carrier. Any sterilant which is a vapor or gas and is

recommended for use in a specific device should be tested using the AOAC International Sporicidal Activity of Disinfectants test in that specific device and according to the directions for use of that specific device. Modifications to the AOAC Sporicidal Activity of Disinfectants test to address this use should be submitted to the Agency for review and approval prior to conducting the tests.

**(2) Evaluation of sterilant success.** The product should kill the test spores on all of the 720 carriers without any failures.

**(e) Validation testing for all products with sterilant claims.** Data submitted to support sterilant claims are subject to independent validation testing in a second laboratory.

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

**(2) Evaluation of sterilant success.** The product should kill the test spores on all 120 carriers without any failures.

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

**(g) Additional spore formers, *Clostridium difficile* (*C. difficile*) claims.** This section addresses interim efficacy tests for products with claims to inactivate *C. difficile* spores on hard, non-porous, inanimate surfaces. The Agency recommends three possible options, as described in paragraphs (g)(1)(i) through (g)(1)(iii) of this guideline.

**(1) Water-soluble powders and liquid products, qualitative testing—(i) Test procedure for sterilant/sporicide plus *C. difficile* claim.** The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method II (Ref. 1) to demonstrate the sterilant efficacy of products, as described in (d)(1). In addition, conduct a **confirmatory test** using the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method I. Until the Agency identifies a representative toxigenic strain or suitable surrogate(s) to be used in testing against *C. difficile*, one of the following toxigenic strains should be used for testing: ATCC 700792, ATCC 43598 or ATCC 43599. *C. difficile* spores are inoculated on thirty carriers (porcelain penicylinders) for two samples, representing two different batches of the product (a total of 60 carriers).

**(A) Evaluation of sporicidal success.** The product should kill all of the test spores on all of the 780 carriers without any failures.

**(B) Reserved.**

**(ii) Test procedure for *C. difficile* sporicides--qualitative testing.** The Agency

recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test, Method I (Ref. 1) using *C. difficile* ATCC 700792, ATCC 43598 or ATCC 43599. Sixty carriers (porcelain penicylinders) should be tested on three samples representing three different batches of product, one of which should be at least 60 days old (a total of 180 carriers). The inoculum employed should provide a target count of  $1 \times 10^5$  – approximately  $1 \times 10^6$  colony forming units per carrier.

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

**(B) Reserved.**

**(iii) Test procedure for *C. difficile* sporicides--quantitative testing.** The Agency recommends the use AOAC Method 2008.05: Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface) (Ref. 2) or ASTM E 2197-02: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides (Ref. 3). Until the Agency identifies a representative toxigenic strain or suitable surrogate(s) to be used in testing against *C. difficile*, one of the following toxigenic strains should be used for testing: ATCC 700792, ATCC 43598 or ATCC 43599. The inoculum employed should provide a target count of  $> 10^6$  colony forming units per carrier. The product should be tested on three samples representing three different batches of product, one of which should be at least 60 days old. The number of carriers will vary depending on the test method.

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

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

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

**(1) Water-soluble powders, liquid products, gases and vapors—(i) Test procedure for sterilant/sporicide plus *B. anthracis* claim.** The Agency recommends use of the Official Methods of Analysis of AOAC International, Official Method 966.04 Sporicidal Activity of Disinfectants test (Ref. 1) to demonstrate the sterilant efficacy of products. Sixty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) should be tested against spores of both *B. subtilis* (ATCC 19659) and *C. sporogenes* (ATCC 3584) on three samples representing three different batches of the product, one of which should be at least 60 days old (240 carriers per sample, or a total of 720 carriers). The inoculum employed should provide a target count of  $1 \times 10^5$  – approximately  $1 \times 10^6$  colony forming units per carrier. In addition, conduct a **confirmatory test** using virulent *B. anthracis* spores (or a surrogate acceptable to EPA) inoculated on thirty carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) on two samples, representing two different

batches of the product (a total of 120 carriers).

**(A) Evaluation of sporicidal success.** The product should kill all of the test spores on all of the 840 carriers without any failures.

**(B) Reserved.**

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

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

**(B) Reserved.**

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

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

**(B) Reserved.**

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

relative humidity and contact time) are accurately monitored and maintained throughout the enclosed space, and establish product generation rate (lbs/hr) and rate/volume (lbs/hr/ft<sup>3</sup>).

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

**(A)** The test should be set up in a sealed enclosure at least the size of a typical office and designed to measure the distribution of the product and conditions needed to meet the measure of success in the laboratory efficacy test. Items that might be treated (e.g., dressers, upholstered furniture, carpet, etc.) during an actual fumigation, should be included in this test.

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

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

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

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

**(iii) Evaluation of sporicidal success.** Measurements should show that the same concentration, temperature, and relative humidity, can be maintained for the required contact time needed to achieve 100% kill (i.e., no growth on any of the carriers) in the qualitative laboratory test, or a 6 log reduction in the quantitative test is demonstrated in the simulated-use test. In addition, measurements of the fumigant mass injection/generation rate (e.g., pounds/hour), divided by the volume of the simulated use test bed, that was used to arrive at the required generation rate/volume (e.g., pounds per hour/cubic foot) for the fumigation, should be included with the data, and listed on the product label.

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

**(i)** Study title;

**(ii)** Product Identity;

- (iii) Guideline number;
- (iv) Identification of the testing laboratory or organization;
- (v) Location where the test was performed;
- (vi) Name(s) of the person(s) responsible for the test;
- (vii) 40 CFR Part 160 Good Laboratory Practice compliance;
- (viii) Purpose of the study;
- (ix) Date and time of the start and end of the test;
- (x) Statistical treatment of the data;
- (xi) Conclusions;
- (xii) References;
- (xiii) Appendices;
- (xiv) Certification

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

**(2) Data Report for recommended methods.** When recommended methods from the Official Methods of Analysis of AOAC International; the Annual Book of Standards of the American Society for Testing and Materials (Ref. 3); or, EPA methods are used to develop efficacy data, certain minimal information, in addition to that described in this guideline, should be provided in the test report. The report should include, at the least, the material in paragraphs (j)(2)(i) through (j)(2)(xii) of this guideline;

- (i) Test employed, and any significant modifications thereto (e.g., organic load, hard water);
- (ii) Test microorganisms employed, including identification of the specific strain (ATCC or other);
- (iii) Description of the test substance, including the percent of active ingredient;
- (iv) Concentration or dilution of the product tested and how prepared;
- (v) Number of samples, batches and replicates tested;

(vi) Manufacture date of each product batch;

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

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

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

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

(xi) Carrier counts;

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

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

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

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

(1) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 966.04 Sporocidal Activity of Disinfectants, Eighteenth edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

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

(3) *Annual Book of ASTM Standards*, Standard Quantitative Carrier Test Method to

Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporocidal Potencies of Liquid Chemical Germicides, Designation E 2197-02. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.