US Environmental Protection Agency
Office of Pesticide Programs

Protocol for Room Sterilization by Fogger Application

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Scope:

This protocol describes an end-use fogging application for sterilizing porous and non-porous surfaces within a sealed and enclosed space. The sterilant will be applied using an acceptable fogging generation system that releases test material (product) into the designated area. This field study will investigate the ability of a sterilant product to kill bacterial spores known to be highly resistant to sterilants and disinfectants via fogging application.

Overview:

This large-scale study protocol will be performed using EPA approved sterilization practices for fogging application of the sterilant product. The fogging generation system used in the sterilization process will achieve the airborne test material concentration for the time period required for sterilization. The distribution of the fog will be assisted with fans. The system should be a completely self contained bi-decontamination system with the ability to dehumidify, generate fog and aerate/decontaminate sealed enclosures. Biological and chemical indicators will be equally distributed throughout the sealed enclosures to allow verification of treatment efficacy. Biological indicators will consist of $\geq 10^6$ *Geobacillus stearothermophilus* spores housed on coupons contained within Tyvek pouches. After treatment, the aeration or decontamination of the sealed enclosures will be performed until the test material is at an acceptable level. Safety monitoring for active ingredient diffusion into adjacent areas will be conducted during the test and in the sealed enclosure after completion of the sterilization process.

Test System:

- Biological indicators (BI) containing $\geq 10^6$ *Geobacillus Stearothermophilus* (ATCC 7953) spores housed onto coupons (0.7 cm X 1.7 cm) contained within Tyvek pouches (1.5 cm X 2.5 cm)
- Six sterile coupons contained within Tyvek pouches
- Fogger Generation System (system must be identified)
- Electrochemical Sensor
- Auxillary Aeration unit
- Dehumidifier device
- Fog/mist Detector
- Oscillating Fan(s)
- Product Solution
- Chemical Indicators (CI)
- Neutralization Media
- Digital Thermohygrometer with memory capacity to store temperature and relative humidity data collected over the entire sterilization cycle
- Data logger able to receive input from the sensor
- A sealed enclosed area (dimensions must be specified). The room may be carpeted with painted drywall ceiling and walls. It can contain items such as a mattress and box spring, upholstered chair, wooden dresser, and wooden nightstand.

Test Acceptance Criteria:

- For testing biological indicators (BI) must contain $\geq 10^6$ *Geobacillus Stearothermophilus* (ATCC 7953) spores.
- All experimental BI(s) must show no growth for *Geobacillus Stearothermophilus*.
- Chemical indicators must demonstrate test material (product) exposure.
- All specified parameters must be met.
- All control BI(s) must show appropriate growth or no growth responses.

Test Procedure:

Inoculum Preparation:

Overnight cultures of *Geobacillus stearothermophilus* grown in nutrient broth are transferred to sporulation agar plates, which consist of nutrient agar containing 1 μg of Mn^{2+}/ml. The plates are incubated at 55°C for 10 days. Spores are collected by flooding the surface of a culture with sterile distilled water and then scraping the surface. The spores collected are washed three times by centrifugation at 8,000 × *g* for 10 min, resuspended in sterile distilled water, and stored at 4°C until they are used. Suspensions may be diluted to provide a target count of $\geq 10^6$ spores per coupon.

Carrier Preparation:

Method of application of inoculum to BI coupon(s) must be specified. The final inoculum level per coupon must contain $\geq 10^6$ Geobacillus Stearothermophilus (ATCC 7953) spores. Inoculated coupons drying procedures and conditions must be described.

Test Agent Preparation:

The test agent will be prepared and used in accordance with the Sponsor's directions and/or proposed label claims.

Test Method:

1. Determine the number of BI(s) required for testing in the sealed enclosed area by using the following formula:

   $[(m^3 - 10) / 2] + 15$, where m^3 is the cubic meter area of the sealed enclosure. Note that this equation is only applicable to enclosures ≥ 60 m^3.

- The BI(s) are placed inside Tyvek pouches to prevent cross contamination. Biological indicators from the same production lot will be used for all testing including controls. Spore populations will be documented from the accompanying BI Certificate of Analysis.

2. Provide a floor plan diagram. Ensure that the placement of materials within the room is in accordance with the provided floor plan.

- List the sealed enclosure dimensions.
- The floor plan must include a diagram of any items located in the room such as carpet, bed, chair, dresser, and nightstand.
- List the position of the BI(s), CI(s), Fogger Generation System, Electrochemical Sensor, Auxillary Aeration unit, dehumidifier device, Fog/mist Detector, Oscillating Fan(s), Digital Thermohygrometer, and Data logger.
- At a minimum, the test locations for the BI(s) must include the following:
    - All corners of the room
    - Various locations on the wall faces
    - Center location on the floor
    - Underneath horizontal surfaces
    - Test locations must include samples at heights above the ground at the maximum height specified in the fogging system instructions.

3. Cover electrical outlets and other fog escape routes with plastic sheeting and tape.

4. Open furniture drawers and doors as well as any interior doors.

5. Use auxillary dehumidifier to dehumidify the sealed enclosure to achieve ≤ 60% relative humidity at ambient temperature (23 ± 5^{o}C) prior to fogging application.

6. Begin the conditioning phase during which the sterilant is injected to reach and maintain the target sterilization phase of product concentration.

- Record all devices identification information used for this procedure.
- Record the time required to achieve the target sterilization phase of product concentration.

7. Once the target sterilization phase product concentration is reached, maintain the injection of the target sterilant concentration for the contact period necessary to sterilize porous and non-porous surfaces.

- The contact time required to achieve 1- log reduction of test spores can be determined by D - values calculations using ASTM E 1891-97, Standard Guide for Determination of a Survival Curve for Antimicrobial Agents Against Selected Microorganisms and Calculation of a D-value and Concentration Coefficient. The D – value may then be used to establish theoretical contact times for achieving 10^{6} log reduction.
- Record the sterilization phase product concentration and the contact period necessary for the sterilization process.

8. After the contact period for the sterilant is completed, initiate the dehumidification phase.

- Areas adjacent to the sealed enclosure must be monitored during the entire process.
- Record the dehumidifier device identification information, time required to dehumidify the sealed enclosure, relative humidity after dehumidification, and temperature.

9. Once the sealed enclosure dehumidification is complete, begin to aerate the sealed enclosure. When the sensor detects the room is at a deemed acceptable product level, it is safe to reenter the enclosed area.

10. Aseptically transfer the exposed BI(s) contained within the Tyvek pouch into sterile containers.

11. Inspect the chemical indicators.

- Verify and record the qualitative information obtained from the CI(s).

12. Process the biological indicators within 24 hours of the conclusion of the aeration phase.

- Incubate the BI(s) with 10 mL of neutralization media for seven (7) days at 55^{o}C.
- After the incubation period, report the results that demonstrated the presence or absence of growth in the BI(s) neutralization media.

Controls:

Sterility- Three sterile coupons in Tyvek pouches are placed within the sealed enclosure during the sterilization cycle. Afterwards, they are transferred to the neutralizing media and incubated at 55^{o}C for seven days in the same manner as the experimental samples.

Neutralization- Three sterile coupons in Tyvek pouches are placed within the sealed enclosure during the sterilization cycle. Afterwards, the coupons are transferred to the neutralizing media. A dilute suspension of a final concentration between 1 and 10 spore(s) per ml of *Geobacillus stearothermophilus* is added to the neutralizing media containing the coupons. The tubes are incubated at 55^{o}C for seven days in the same manner as the experimental samples.

Viability- Three inoculated BI(s) not exposed to the sterilant are transferred to the neutralizing media and incubated in the same manner as the experimental samples to serve as a comparison for the test coupons.

Microbial populations on BI(s)- The initial population of spores on the coupons will be confirmed by enumeration prior to the study. Transfer a BI into a tube containing 10 mL of nutrient media. Sonicate each tube containing the BI for 10 minutes and then vortex for 5 seconds. Perform serial dilutions of the sonicated suspension in sterile deionized water and enumerate using the pour plate method. Incubate the plates at 55^{o}C for 48 hours and enumerate.