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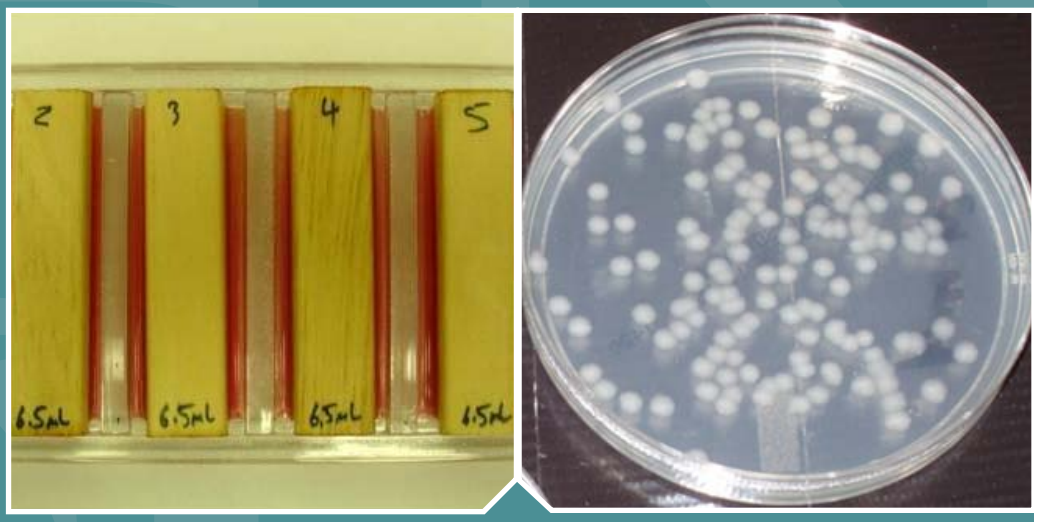
United States
Environmental Protection
Agency

Systematic Investigation of Liquid and Fumigant Decontamination Efficacy against Biological Agents Deposited on Test Coupons of Common Indoor Materials

TECHNOLOGY EVALUATION REPORT



Office of Research and Development
National Homeland Security Research Center

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Investigation and Technology Evaluation Report

Systematic Investigation of Liquid and Fumigant Decontamination Efficacy against Biological Agents Deposited on Test Coupons of Common Indoor Materials

United States Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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Foreword

Following the events of September 11, 2001, addressing the critical needs related to homeland security became a clear requirement with respect to EPA's mission to protect human health and the environment. Presidential Directives further emphasized EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological (CBR) attack. To support EPA's mission to assist in and lead response and recovery activities associated with CBR incidents of national significance, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of the Agency and other federal, state and local agencies to carry out their homeland security responsibilities.

One goal of NHSRC's research is to provide information on decontamination methods and technologies that can be used in the response and recovery efforts resulting from a CBR release over a wide area. The complexity and heterogeneity of the wide-area decontamination challenge necessitates the understanding of the effectiveness of a range of decontamination options. In addition to effective fumigation approaches, rapidly deployable or readily available surface decontamination approaches have also been recognized as a tool to enhance the capabilities to respond to and recover from such an intentional CBR dispersion.

Through working with ORD's program office partners (EPA's Office of Emergency Management and Office of Chemical Safety and Pollution Prevention) and Regional on-scene coordinators, NHSRC is attempting to understand and develop useful decontamination procedures for wide-area remediation. This report documents the results of a laboratory study designed to better understand the effectiveness of several fumigation and liquid decontamination methods for materials contaminated with biological agents. The primary focus of the work is relevant to *Bacillus anthracis* spores; some data is alos presented related to smallpox virus and ricin toxin.

These results, coupled with additional information in separate NHSRC publications (available at www.epa.gov/nhsrc) can be used to determine whether a particular decontamination technology can be effective in a given scenario. NHSRC has made this publication available to the response community to prepare for and recover from disasters involving chemical and/or biological contamination. This research is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
National Homeland Security Research Center

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Executive Summary

The U.S. Environmental Protection Agency (EPA), Office of Research and Development is striving to protect human health and the environment from adverse impacts resulting from acts of terror by investigating the effectiveness and applicability of technologies for homeland security (HS) related applications. The purpose of this investigation is to determine the efficacy of various fumigation and liquid decontamination technologies for inactivating biological threat agents (spore, virus, and biotoxin) as a function of building material surface and decontamination parameters (concentration, contact time, temperature, and percent relative humidity [RH]). The objective of these studies is to provide an understanding of the performance of technologies or decontamination methods to guide scenario-specific selection and implementation of technologies of HS applications. In the assessment of options for decontamination of indoor surfaces following intentional release of biological agents, it is important to know whether and to what extent such factors can impact the decontamination efficacy. This investigation focused on decontamination of indoor surfaces typical of those found in public buildings or transportation facilities with the ultimate goal of restoring the buildings or transportation facilities to a usable state using appropriate decontamination technologies.

Bacillus anthracis spores, ricin toxin, and vaccinia virus were selected for this investigation based upon a review of available information and other ongoing research and assessment efforts; the selection represents a range of biological agents with an expected range of ease of decontamination. *B. anthracis* spores are the causative agent for anthrax and are the most difficult to kill of the selected agents. Ricin is an extremely toxic protein extracted from castor beans (*Ricinus communis*). Vaccinia is the virus used for vaccination against smallpox.

The building materials included in the investigation were: glass (small), painted concrete block, decorative laminate, blown cellulose insulation, particle board, industrial carpet, plate glass, painted I-beam steel, unpainted pine wood, and ceiling tile. Not all materials were used with every biological agent or decontamination method. Temperatures selected for use in testing ranged from 20 °C to 37 °C.

Decontamination technologies investigated included three fumigant technologies (chlorine dioxide [ClO_2], methyl bromide [MeBr], and hydrogen peroxide [HP]) and four liquid decontamination technologies (pH-amended bleach, ClO_2 solution, and two different HP/peracetic acid [HP-PA] solutions).

Summary of Fumigation Results

The ClO_2 fumigation results showed efficacy against *B. anthracis* spores, ricin toxin, and vaccinia virus. The efficacy was dependent on the type of materials onto which the spores were inoculated and the strain of *B. anthracis*. ClO_2 fumigation at a concentration x time (CT) of 1000 ppmv-hour (hr) resulted in no *B. anthracis* colony-forming units (CFU)

being recovered from most types of coupons (>7 log reduction). The mass of ricin remaining on coupons after ClO_2 fumigation at a CT of 500 ppmv-hr of ClO_2 was reduced by >99.1% (geometric mean) from all materials tested except cellulose insulation which exhibited a 92.7% reduction in recovered mass of ricin compared to controls. No viable vaccinia virus was recovered from any coupon of any of the seven materials tested after fumigation at a target CT of 125 ppmv-hr of ClO_2.

MeBr fumigation demonstrated up to complete kill of *B. anthracis* spores (>6 log reduction in CFU, no CFU recovered) dependent on the building material, fumigant concentration, and contact time. *B. subtilis* is a surrogate for the virulent Ames strain of *B. anthracis* that was included in the MeBr fumigation tests. Little or no efficacy of MeBr against *B. subtilis* was observed in the CT range or 1260–1899 mg/L-hr. In contrast, a high level of MeBr efficacy was observed against *B. anthracis* Ames spores at CTs of 1575 mg/L-hr or greater; no viable *B. anthracis* spores were recovered at CTs of 1890 mg/L-hr (105 mg/L for 18 hr) or 1899 mg/L-hr (211 mg/L for 9 hr).

Decontamination at alternative RH was investigated for ClO_2 and MeBr. Both ClO_2 and MeBr showed efficacy to be impacted by the RH of the test chamber. Specifically, fumigation at higher humidity (80%–84% for ClO_2 and 75% for MeBr) exhibited higher efficacy against *B. anthracis* Ames spores than fumigation at lower humidity (71%-77% for ClO_2 and 40% for MeBr).

HP fumigation demonstrated up to >6 log reduction in *B. anthracis* Ames spores (no CFU recovered) dependent on the building material and contact time. The HP concentration was 500 ppmv for all fumigations. The CT required for a 6-log reduction in recoverable *B. anthracis* Ames spores appears to depend on the type of building material.

Summary of Liquid Decontamination Results

All liquids were applied by immersing the coupons directly into the decontaminant. This approach may be in contrast to actual field application and other results may suggest difference due to application procedures (e.g., spray application, which was also evaluated by EPA).[1]

Bleach adjusted to pH 7 provided complete kill of *B. anthracis* Ames spores (0 CFU recovered, >6 log reduction) dependent on the building material and the contact time. The efficacy of pH-amended bleach against three strains of *B. anthracis* spores (virulent Ames and avirulent Vollum and NNR1Δ1) and *B. subtilis* was evaluated using the quantitative method. The log reduction in recovery of various types of viable *Bacillus* spores on coupons exposed to pH-amended bleach or phosphate buffer solution for specified time periods was evaluated. In addition, the extracted coupons were placed individually into tryptic soy broth (TSB) and incubated for seven days to look for cloudiness, indicating the presence of residual viable spores on the test coupon. In all cases where no spores were observed using the quantitative methodology, no residual viable spores were detected using the qualitative method. While strong similarities in efficacy were observed across spore types, differences were also observed. For example, viable *B. anthracis* Vollum and *B. subtilis* spores were present on painted concrete after

exposure to pH-amended bleach for five minutes (min); the other two strains of *B. anthracis*, Ames and NNR1Δ1, did not have viable spores present after exposure to pH-amended bleach for 5 min.

Exterm (brand) ClO_2 solution provided >6 log reduction in recovered viable *B. anthracis* Ames spores dependent on the building material and the contact time. In general, *B. subtilis* spores appear to be able to survive a longer exposure to ClO_2 solution than *B. anthracis* Ames spores.

Spor-Klenz® hydrogen peroxide-peracetic acid (HP-PA) solution exhibited a range of efficacies, up to >6 log reduction in *B. anthracis* Ames spores (no viable spores recovered), dependent on the building material and contact time. No viable *B. anthracis* Ames or *B. subtilis* spores were recovered from any glass coupons after a 20-30 min contact time. A complete kill of spores on decorative laminate, i.e., no viable spores recovered or detected, occurred at a 30-min contact time with Spor-Klenz® HP-PA for *B. anthracis* Ames spores. In contrast, less than a 2-log reduction in viable spores was observed after a 30-min contact time with Spor-Klenz® HP-PA for *B. anthracis* Ames spores on galvanized metal.

Oxonia Active® HP-PA solution exhibited a range of efficacies, up to >6 log reduction in *B. anthracis* Ames spores (no viable spores recovered), dependent on the building material and contact time. Oxonia Active® HP-PA against *B. anthracis* Ames and *B. subtilis* spores, reported as log reduction, showed similar efficacies. For both *B. anthracis* Ames and *B. subtilis* on glass, a >6 log reduction was observed after a 10-min contact time (except that only a 5.77 log reduction was observed for *B. subtilis* after 60 min). Small numbers of viable spores were recovered from one or more replicate coupons of some material for both *B. anthracis* Ames and *B. subtilis* at each contact time (10, 30, and 60 min).

Table of Contents

Figures

Tables

Abbreviations/Acronyms

AOAC	Association of Official Analytical Chemists *(now AOAC International)*
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BBRC	Battelle Biomedical Research Center
BI	biological indicator
BSC II	Class II biological safety cabinet
BSC III	Class III biological safety cabinet
^{o}C	degrees Celsius
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	colony-forming unit(s)
ClO_2	chlorine dioxide
cm	centimeter
CO_2	carbon dioxide
CT	concentration x contact time
d	depth
ECBC	U. S. Army Edgewood Chemical Biological Center
EPA	U.S. Environmental Protection Agency
H	height
HP	hydrogen peroxide
HP-PA	hydrogen peroxide – peracetic acid
hr	hour
HS	homeland security
HVAC	heating, ventilation, and air conditioning
l	length
L	liter
mL	milliliter
µg	microgram
µL	microliter
min	minute
mm	millimeter
mg	milligram

MTT	3-(4,5-dimethylthiazol-2-yl)-2, 5,-diphenyl tetrazolium bromide
nm	nanometer
NIST	National Institute of Standards and Technology
ORD	Office of Research and Development
PBS	phosphate-buffered saline
PE	performance evaluation
PFU	plaque-forming unit(s)
ppm	parts per million
ppmv	parts per million volume
psi	pounds per square inch
PSR	perfectly stirred reactor
QA	quality assurance
QC	quality control
RH	relative humidity
rpm	revolutions per minute
SD	standard deviation
TSA	trypitic soy agar
TSB	tryptic soy broth
T0	time zero
µg	Microgram
vs.	versus
w	width

1.0 Introduction

The U.S. Environmental Protection Agency, through its Office of Research and Development (ORD), is helping to protect human health and the environment from adverse impacts resulting from intentional acts of terror. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, EPA is working to develop tools and information that will help detect the intentional introduction of chemical, radiological, or biological contaminants in buildings, subways, water systems, or outdoor environments; contain these contaminants; decontaminate buildings, subways, water systems, and/or outdoor environments; and facilitate the disposal of material resulting from cleanups.

As part of the above effort, EPA investigates the effectiveness and applicability of technologies for homeland security (HS) related applications by developing test plans that are responsive to the needs of stakeholders, conducting tests, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous QA protocols to ensure that data of known and high quality are generated and that the results are defensible. EPA provides high-quality information that is useful to decision makers in purchasing or applying the tested technologies. EPA provides potential users with unbiased, third-party information that can supplement vendor-provided information. Stakeholder involvement ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies.

The purpose of this investigation was to develop an understanding of the effectiveness of decontamination approaches for HS applications as a function of a range of materials, environmental conditions, and operating conditions typical of field use. This investigation focused on decontamination of indoor surfaces typical of those found in a public building and public transportation that could be contaminated by biological agent following an intentional release. Residual amount of biological agent on indoor surfaces following decontamination after an intentional release could present a potential health risk for personnel re-entering the building. This is a report of the impact of various factors on the efficacy of fumigant and liquid decontamination technologies against biological warfare agents and surrogates. (Subsequent use of the term “biological agents” should be understood to include surrogates.)

The investigation generated data that are indicative of the performance or efficacy of the fumigation and liquid decontamination technologies under a variety of parametric conditions, including non-ideal conditions. This

bench scale investigation can be summarized as follows. Biological agents were applied to a variety of test coupons representative of porous and non-porous indoor building surfaces. A variety of fumigation and liquid decontamination technologies were applied under conditions that were expected to be highly effective as well as conditions that might be partially effective. The factors that were varied included: duration of contact with the decontamination technology (contact time), concentrations of the fumigant decontamination technologies, temperature, and RH (for fumigation technologies). Residual biological agent was extracted from the coupons and a quantitative measurement was made of the viable organisms or level of cytotoxicity. Efficacy of the decontamination technology under a given set of conditions was evaluated by comparing the residual biological agent after the decontamination treatment to a measurement of biological agent extracted from positive control coupons incubated under environmental conditions specified for a test and for the same time period. (Various factors were varied for different technologies.)

All testing and evaluation were conducted under the direction of the EPA. In performing each test, peer-reviewed test/QA plans and amendments were developed, approved by EPA, and followed in execution of the testing.

Chapter 2 summarizes the methods used in this investigation. Chapter 3 provides information on the quality assurance and quality control methods that were employed and a summary of quality audits and deviations. Chapter 4 provides the results of the fumigation testing. Chapter 5 provides the results of liquid decontamination testing.

2.0 Investigation Procedures

This section provides an overview of the investigation procedures that were used. For all tests, specific test plans detailing the test methodology and data quality assurance provisions were developed prior to testing. Important aspects of those test plans are provided in this section (test plan details) and Section 3.0 (quality assurance documentation).

2.1 Experimental Design

This report provides results for the bench-scale investigation of technologies to decontaminate biological agents from indoor surfaces. The general approach and methods, biological agents, and types of indoor surfaces used are summarized in this section. A pretest-posttest control group design was used. The independent test parameters include the type of biological agent, type of building material, type of decontamination technology, contact time, concentration of the decontamination technology, temperature, and RH. The dependent variable is the number of viable organisms or amount of bioactive toxin extracted from a test or positive control coupon with or without exposure to the decontamination technology.

Key measurements in this investigation include:

- Quantitative measurement of viable organisms or level of bioactivity
- Environmental conditions including temperature and RH
- Operating conditions including contact time with the decontamination technology and, for fumigants, concentration of the decontamination technology.

The key outputs of this investigation are concentration x contact time (the product henceforth referred to as CT) versus (vs.) log reduction curves, showing the efficacy:

- Of the various technologies
- Against various biological warfare agents
- On various indoor building surfaces
- Under various environmental conditions.

Statistical comparisons were conducted to determine whether the mean log reduction in viable biological agent or bioactive toxin exceeded the control mean log reduction by an amount that was statistically significant at a 95% confidence level.

2.2 Test and Apparatus

Decontamination was performed by placing coupons into customized test chambers and applying the fumigation

technology, or by immersing the coupons in liquid decontamination technologies either in a custom-built trough system or capped vials. For positive controls, corresponding control chambers, troughs, or capped vials were used to replicate the conditions in the test chambers and troughs, but precluding contact with the decontamination technology.

2.2.1 Fumigation Test and Control Chambers

The impact of the critical parameters on decontamination efficacy of various fumigation technologies was evaluated through bench-scale testing in the laboratory. The fumigation test chambers were a 317 L glove box for ClO_2, a 23 L glass chamber for MeBr, and a 1275 L Class III biological safety cabinet (BSC) for HP fumigation. The glass chamber was needed for MeBr because the gas exhibits high penetration through many materials.

The glove box was a Compact Glove Box Model 830-ABC (Plas Labs, Inc., Lansing, MI), shown in Figure 2-1, with internal dimensions of 71 cm width (w) x 59 cm diameter (d) x 74 cm height (h) and external dimensions of 110 cm (w) x 61 cm (d) x 79 cm (h), having a total volume of 317 L. The glove box also has a top opening of 43 cm x 58 cm and an attached transfer chamber that was 30 cm long and an inner diameter of 28 cm. For ClO_2 investigation, the glove box is coated with black latex paint to shield the interior from light. Glove ports enabled working in the glove box.

For positive control (spiked with biological agent) and laboratory blank coupons (not spiked with biological agent) that were not exposed to ClO_2 fumigation, an identical Compact Glove Box Model 830-ABC (not painted black) was used.

As was done in previous studies,[2] multiple coupons of each indoor material were spiked with the biological agent and placed horizontally on a wire rack the test chamber. For ClO_2 investigation, the glove box was coated with black latex paint to shield the interior from light. Two 93 mm computer fans in the glove box provided air flow in the test chamber to promote uniform exposure to the fumigant throughout the chamber; identical fans were included in the control chamber. The short-term stability of the ClO_2 measurement in the circulating air suggests that mixing was sufficient to provide uniform exposure. Blank (i.e., uncontaminated) and positive control (i.e., contaminated but not decontaminated) coupons were also prepared for each test material, and were utilized along with data from the test contaminated and decontaminated (test) coupons to determine decontamination efficacy. This approach provides a highly controlled, reproducible approach to assess sensitivity of the fumigation decontamination efficacy to CT, temperature, and RH.

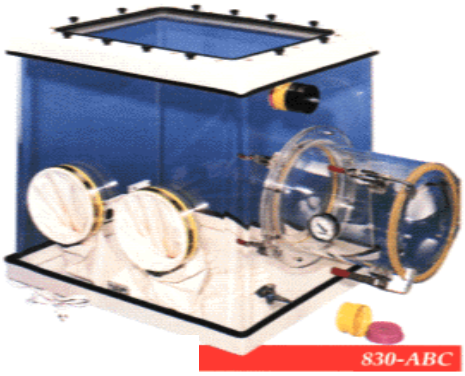


Figure 2-1. Glove box used for test and control chambers for ClO_2 fumigation testing.

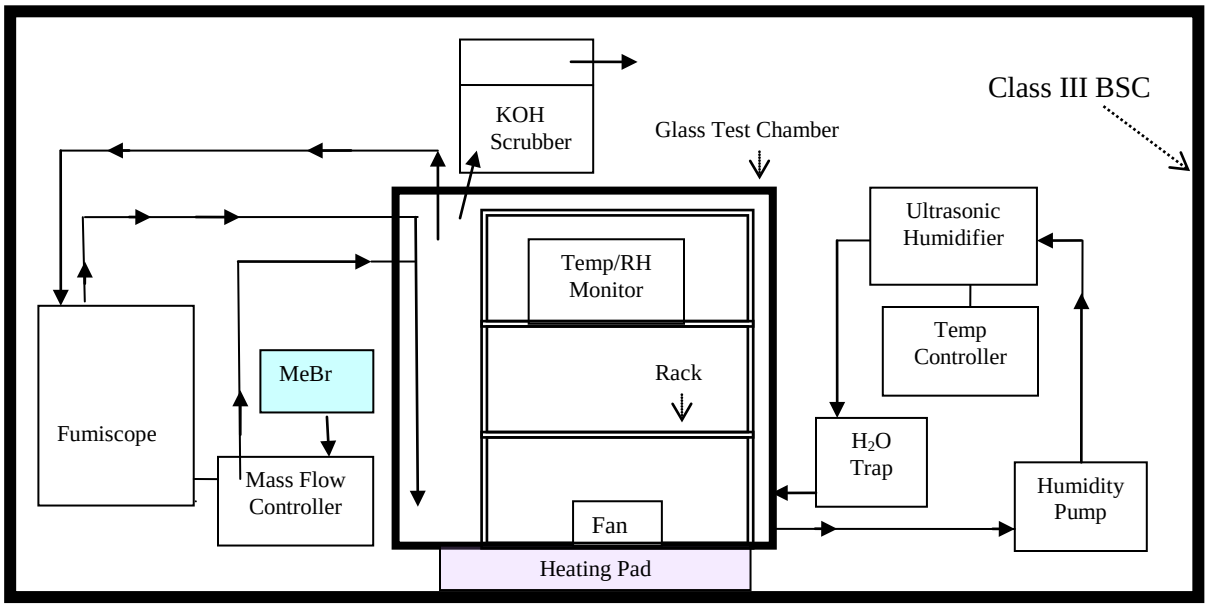


Figure 2-2. Schematic of MeBr decontamination testing.

Figure 2-2 shows a schematic drawing of the MeBr test chamber and containment system. The primary test chamber was glass with a 23 L volume (approximately 29 cm x 29 cm x 29 cm). A second chamber of the same size made of polycarbonate plastic was used as the control chamber. The chambers were insulated to prevent condensation on the inside chamber walls. The high toxicity and penetrability of MeBr required a primary and secondary containment chamber for protection of laboratory personnel. A Class III biological safety cabinet (BSC III) (SG603, Baker, Sanford, ME) provided secondary containment.

For evaluation of the HP fumigation, a BSC III was used as the fumigation test chamber having a total volume of approximately 1275 L. Positive control coupons were in sealed vials within the

fumigation test chamber during the fumigation cycle.

The experimental temperature and RH in test chambers and the control chambers were monitored and, where specified in the test plan, controlled during a given fumigation event ("trial"). Temperature and RH were manually recorded at defined intervals using a calibrated, National Institute of Standards and Technology (NIST)-traceable thermometer/hygrometer (accuracy of ±1 °C and ±5% RH; 14-648-53, Fisher Scientific, Pittsburgh, PA).

As testing proceeded, decontamination efficacy was greater at high RH than at low RH; however, moisture was observed on surfaces at high RH. Because moisture may absorb the ClO_2 from the atmosphere, the fumigation at high RH may become a liquid decontamination technology. To address this concern, the RH requirements were revised and tolerances were tightened (75% ± 5%). In the early ClO_2 fumigation testing, where broad RH levels were specified (<40% RH, >75% RH), low RH was maintained in the chambers using calcium sulfate desiccants (W.A. Hammond Drierite Co., Xenia, OH). The W.A. Hammond Drierite Co. web site (www.Drierite.com) reports that the National Bureau of Standards has verified that the moisture remaining in gases dried with Drierite at 25 °C-30 °C is 0.005 mg/L. High RH in the test chamber during the ClO_2 fumigation was generated using a nebulizer approach previously used to maintain high humidity during testing. High RH in the control chamber was achieved by enclosing a wet paper towel.

Based on knowledge gained during testing, the RH tolerances were tightened (e.g., 75% ± 5%) and methods for adjusting humidity were modified to prevent possible generation and deposition of moisture (liquid water) in the test and control system. To meet these tighter requirements, a custom-designed ultrasonic fogging system with a water trap, shown connected to the MeBr chamber in Figure 2-3, was developed and used to humidify the test chamber. The ultrasonic fogger was inside a polyvinylchloride pipe that was surrounded by heating tape and covered with insulation. A water sight gauge allowed the water level to be monitored. Humidified air from the fogging system, at the specified temperature for the trial, was pumped through a water trap to remove liquid water and flushed through the test chamber until the specified RH was reached. The system can be operated in a single-pass mode or the test chamber air can be recirculated through the fogging system. During method development, Water Contact Indicator Tape (3M, St. Paul, MN) was used to show that the humidified air introduced into the test chamber did not condense into liquid water. The RH was lowered by introduction of dry air. This approach provided tightly controlled RH when the chamber was static and allowed adjustment of RH when the chamber atmosphere was dynamic, e.g., during introduction of the fumigant.

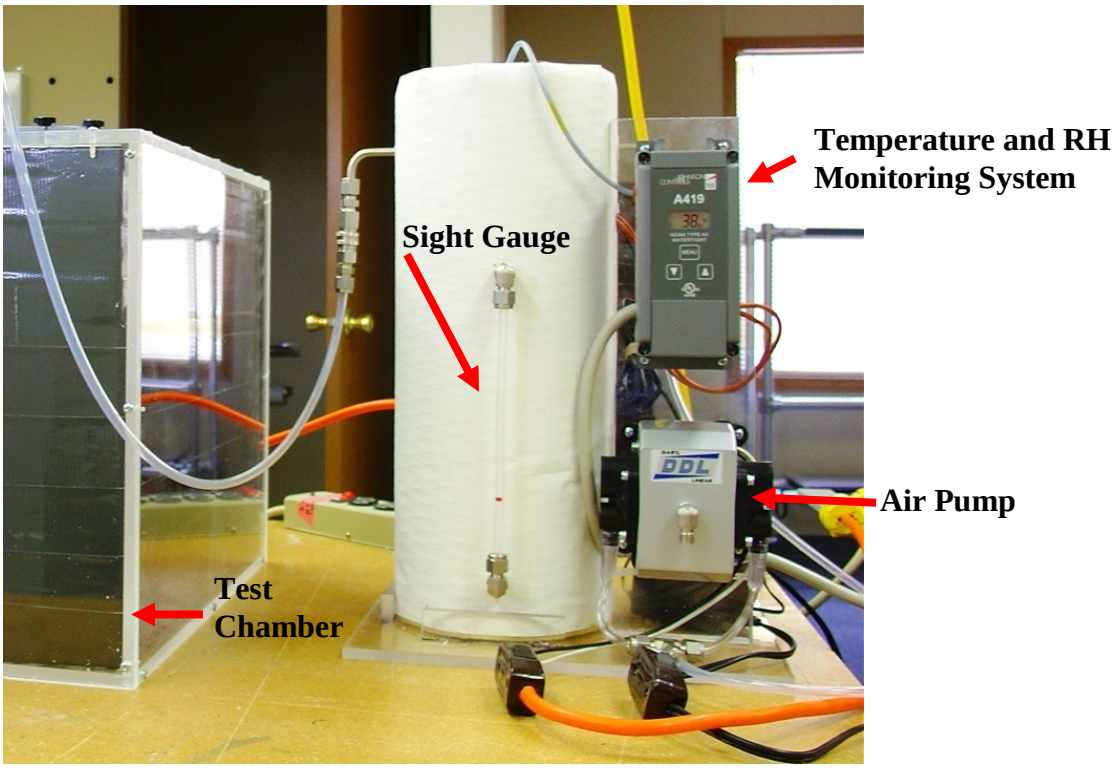


Figure 2-3. Ultrasonic fogging system.

Temperatures of all chambers above ambient, except the MeBr control chamber, were regulated using a thermostatically-controlled heating pad. The MeBr control chamber was placed in an incubator to maintain the temperature. When the RH tolerances were tightened, the MeBr test chamber was insulated to help maintain a constant temperature and to prevent any potential condensation. The MeBr control chamber was in an incubator; insulation was not necessary.

2.2.2 Apparatus for Testing Liquid Decontamination Technologies

The liquid decontamination testing was performed by immersing the coupon surface spiked with biological agent in the liquid decontamination technology. The immersion of the contaminated surface in the liquid decontamination technology provides a well-controlled, conservative efficacy test. Spraying of the liquid decontamination technology onto contaminated materials may yield different results. For the pH-amended bleach and Exterm liquid ClO_2 testing, the test coupons were placed in custom-built trays (shown in Figure 2-4). The custom tray in Figure 2-4 consists of six wells separated from each other by walls. Note: In Figure 2-4, the spiked surfaces of the numbered wooden coupons are facing downward. In this position the spiked surface was in contact with the decontamination fluid. The wells were deep enough to hold one coupon and enough decontamination liquid to cover the bottom surface of the coupon without overflowing into adjoining wells. The specific amount of decontamination liquid required in the wells to ensure continuous contact with each material type during

decontamination was determined. During the testing, the spiked surface remained in contact with the liquid decontaminant for the decontamination period and at the temperature specified in the test/QA plan.

For the Spor-Klenz® and Oxonia Active® testing, test coupons and procedural blanks were placed into 50 mL conical vials (21008-714, VWR, West Chester, PA) holding enough decontamination liquid to cover the surface of the coupon for the specified decontamination period. The spiked surface remained in contact with the decontamination technology liquid for the decontamination period and at the temperature specified in the test/QA plan.

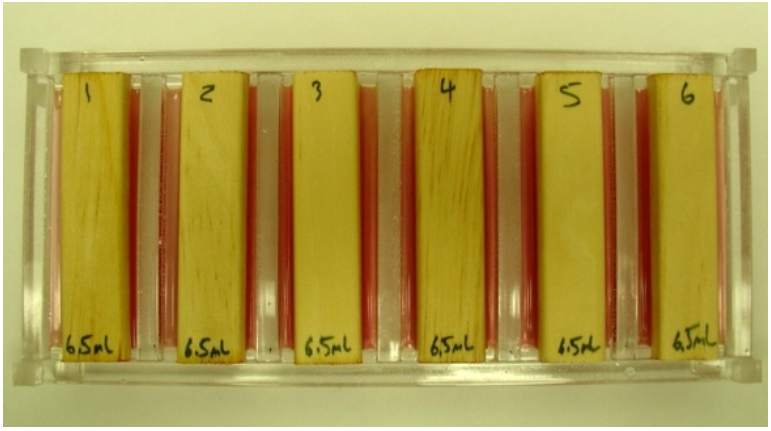


Figure 2-4. Custom apparatus for immersing coupon surface in liquid.

2.3 Test Surfaces

The indoor building material test coupons used in the evaluations are described in Table 2-1. Generally, the indoor building material test coupons were cut from the interior of a large piece of test material to a standard size of approximately 1.9 cm (w) x 7.5 cm length (l). Edges and damaged areas were avoided in cutting test coupons. The thickness of the coupons varied as shown in Table 2-1. The test coupons were visually inspected prior to being spiked with the biological agents. Coupons were visually inspected for irregularities and those with anomalies were rejected.

Some types of coupons varied from the standard size. The small glass coupons were 5 mm x 5 mm and were of the size and type specified in the EPA's modified Three Step Method for evaluating decontamination efficacy.[3] The small glass coupons both represent glass that was found in buildings and provided a linkage to the Association of Official Analytical Chemists (AOAC) 2008-05[3] method for evaluating decontamination efficacy. The data enable comparison of results from this investigation with results using the AOAC 2008-05 method, controlling for coupon material and size that could result in differences in efficacy results attributable to the specific test method used. Such comparisons are useful to inform

decisions by the EPA Office of Pesticide Programs.

Coupons of compressed blown cellulose insulation were round with a 13 mm diameter. Unpainted wood and painted I-beam steel coupons were 1.3 cm x 1.3 cm and were provided by the U. S. Army Edgewood Chemical Biological Center (ECBC). The latter materials represent materials found in buildings and provide a linkage between the decontamination testing reported here and decontamination testing performed by EPA/ECBC. These linkages enable evaluation of whether comparable efficacy results are obtained by the two laboratories. Knowing of potential interlaboratory differences is important for interpreting results, identifying additional key variables, and standardizing test methods.

On each day of testing, each large coupon was assigned and marked with a unique identifier code for traceability. Small coupons were also assigned a number and placed into containers marked with the unique identifier code. To prevent contamination of test surfaces, sterile technique, following developed policies and guidelines was exercised during all phases of handling the coupons.

2.4 Biological Agents and Surrogates

The biological agents used in the testing include:

- *Bacillus anthracis* Ames (produced from BAA365 seed stock received from the U.S. Army Medical Research Institute for Infectious Diseases)
- *Bacillus anthracis* Vollum strain received from a confidential source and maintained in culture by Battelle (strain verified by genotyping by independent laboratory)
- *Bacillus anthracis* NNR1Δ1 with 0.5% bovine serum albumin (as prepared and provided by the U.S. Army ECBC)
- *Bacillus subtilis* on stainless disk in Tyvek® pouches (Apex Laboratories, Apex, NC) biological indicators
- *Bacillus atrophaeus* spores on filter paper strips (spore strip) in glassine envelopes (Raven Biological Laboratories, Omaha, NE) biological indicators
- Ricin toxin (Vector Laboratories, Burlingame, CA, L-1090 from Vector Laboratories, product specification: *Ricin communis* agglutinin II, 5 mg/mL protein concentration)
- Vaccinia virus (American Type Culture Collection test VR119).

The biological agents were selected based on an evaluation of potential threats to buildings and discussions with and approval by EPA. Each biological agent was used according to the Centers for Disease Control and Prevention (CDC) Select Agents Program (42 CFR Part 73) and the Biological Defense Research Program (32 CFR 626 and 627) in adherence with the Battelle Biomedical Research Center (BBRC) Facility Safety Plan safety plans.

Table 2-1. Material Characteristics

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name	Approximate Coupon Size, w x l (thickness)	Material Preparation
Glass	Same as in AOAC 2008.05	Erie Scientific Company, Portsmouth, NH	5 mm x 5 mm (1 mm)	Autoclaved
Painted Concrete Block	ASTM C90	Wellnitz, Columbus, OH	1.9 cm x 7.5 cm (5 mm)	Brush and roller painted all sides. One coat Martin Senour latex primer (#71-1185) and one coat Porter Paints latex semi-gloss finish (#919); autoclaved
Galvanized Metal Ductwork	Industry HVAC standard 24 Gauge Galvanized Steel	Accurate Fabrication, Columbus, OH	1.9 cm x 7.5 cm (1 mm)	Cleaned with acetone; autoclaved or gamma irradiated
Decorative Laminate	Laminate/ Formica/ White Matte Finish	Solid Surface Design, Columbus, OH	1.9 cm x 7.5 cm (1 mm)	Gamma irradiated
Blown Cellulose Insulation (compressed)	Cocoon Attic Blow-in Insulation Batch 06 10-29-04 15-23	U.S. GreenFiber, LLC Charlotte, NC	13 mm diameter containing 100 mg of material (1 mm)	Compressed at 2000 psi in a mold; gamma irradiated
Particle Board	Appearance is pressed medium density fiberboard	Weyerhaeuser	1.9 cm x 7.5 cm (1.3 cm)	Gamma irradiated
Industrial-Grade Carpet	ShawTek, EcoTek 6 Color: mottled gray/dark brown/and black (or equivalent)	Shaw Industries, Inc. (or equivalent)	1.9 cm x 7.5 cm (7 mm)	Gamma irradiated
Plate Glass	C1036, 1/8" thick	Brooks Brothers	1.9 cm x 7.5 cm (3 mm)	Autoclaved
Painted I-beam Steel	ECBC	ECBC	1.3 cm x.1.3 cm	Autoclaved
Unpainted Pine Wood	ECBC	ECBC	1.3 cm x.1.3 cm	Autoclaved
Ceiling tile	Armstrong 954, Classic Fine Textured (or equivalent)	Armstrong (or equivalent)	3.5 x 1.5 cm	Gamma irradiated

The *B. anthracis* Ames and Vollum spores were prepared according to established BBRC procedures. Details of the method are published in the Journal of Applied Microbiology.[2] Preparations have >95% refractile spores with <5% cellular debris. No final additives were included. Stock spore suspensions (approximately 1.0 x 10^9 CFU/mL) are stored at 2 °C to 8 °C until use. No additives were included in the final spore preparations.

The *B. anthracis* NNR1Δ1 spores were used in the stock suspension as received.

The biological indicators (BIs), *B. subtilis* spores on stainless steel and *B. atrophaeus* spores on filter paper strips, were used as received or with the packaging removed. Biological indicators are typically used without removal from their packaging. Efficacy testing was performed both using the biological indicators enclosed in the glassine or Tyvek® packaging and with the packaging removed to allow a comparison of the impact of the packaging on the surrogate efficacy results.

Ricin was used in the stock solution as received.

Vaccinia virus from Battelle stock was propagated in Vero cells following Battelle's internal methods (BBRC Method No. 116-03/Microbiology, Monkeypox and Vaccinia Plaque Assay and BBRC Method No. 107-04/Microbiology, Method for the Routine Maintenance of Multiple Adherent Cell Lines). A monolayer of Vero cells was inoculated with vaccinia virus at a multiplicity of infection ranging from 0.01 to 1.0%. Cultures were maintained in an incubator at 37 °C ± 2 °C under a 95% air, 5% CO_2 mixture until 90%-100% cytopathic effects were observed. Infected cells were harvested and subjected to a rapid freeze/thaw cycle. Cellular debris was removed by centrifugation at 800 to 1,000 x g. The resulting supernatant (containing virus) was harvested and stored in 1.0 mL aliquots, approximately 0.7 x 10^8-1.0 x 10^8 PFU/mL, at ≤ -70 °C until use. The resulting supernatant, vaccinia in complete cell culture medium containing 5%-10% fetal bovine serum, was not filter sterilized since the virus was propagated in sterile tissue culture. All manipulations were performed with sterile technique. The PFU/mL was determined after the virus was aliquoted by randomly selecting and assaying vials of the stock samples. The samples were assayed using a standard plaque assay (see Section 2.6.3) to determine the viral titer of the entire lot.

2.5 Application of Biological Agents to Test Coupons

Application of *B. anthracis* (Ames, Vollum, or NNR1Δ1) spores was performed in a BSC III. The large test coupons (1.9 cm x 7.5 cm) were placed lying flat in the cabinet and contaminated at challenge levels of approximately 1 x 10^8 CFU of spores per coupon. A 100 µL aliquot of a stock suspension (approximately 1 x 10^9 CFU/mL) of organisms was dispensed as shown in Figure 2-5 using a multi-channel micropipette applied as two rows of five 10 µL droplets across the surface of all the test coupons except for the small glass, blown cellulose insulation, and ECBC coupons. The small glass and blown cellulose insulation coupons were spiked by a single 10 µL drop containing about 10^7 spores of biological agent or surrogate. The unpainted pine and painted I-beam coupons (1.3 cm x 1.3 cm) from ECBC were spiked with seven-7.1 µL aliquots (49.7 µL total) of a stock suspension of known concentration of *B. anthracis* (Ames or NNR1Δ1) spores (1.0 x 10^9 CFU/mL). The 50 µL spike volume matched the spike volume used by ECBC in their tests. (Because results from the testing at Battelle were being

compared to the testing by EPA/ECBC to look for potential causes of differences in test results, potential sources of variation between the laboratories, such as volume applied to coupons, were controlled.) The application of multiple droplets is consistent with other testing reported here, except that only seven droplets, rather than 10 droplets, were used because of the small coupon surface. The coupons spiked with spores were dried overnight in a BSC III cabinet at 22 °C ± 2 °C and 40% ± 10% RH. The temperature and RH at the beginning and end of the drying period were documented.

The ricin solution was applied onto the test coupons, lying flat, at approximately 25 µg per test coupon. A 5 µL aliquot of a stock suspension (5 mg/mL of ricin) was dispensed using a micropipette as a streak across the surface of the test coupon. The ricin application was performed in a Class II BSC. The ricin coupons were left undisturbed in an appropriate BSC cabinet or container at ambient laboratory temperature and RH (22 °C ± 2 °C and 40% ± 10% RH) for 1 hr before use in technology evaluations.

Application of vaccinia virus to test coupons was performed in a Class II BSC. Test coupons were placed lying flat in the cabinet and contaminated at challenge levels of approximately 1 x 10^7 PFU of vaccinia virus per coupon. A 100 µL aliquot of a stock suspension (approximately 1 x 10^8 PFU/mL) of organisms was dispensed using a multi-channel micropipette applied as two rows of five 10 µL droplets across the surface of all the test coupons except glass. The small glass coupons were spiked by a single 10 µL drop containing a specified total amount of biological agent or surrogate. The vaccinia coupons were immediately transferred into the test chamber for fumigation.

Except as specified in a given trial, no additional organisms or organic materials (organic burden) were added to the suspensions and stock solutions of agents and surrogates before application to the coupons.

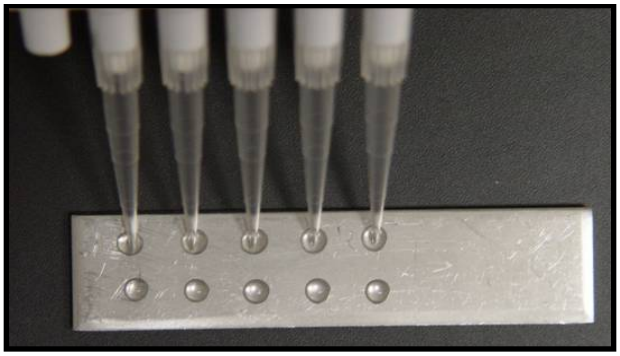


Figure 2-5. Multichannel micropipette applying biological agent to a coupon.

2.6 Extracting and Quantifying Biological Agent and Surrogates (Quantitative Method)

Aeration followed by dilution in an extraction buffer was used to terminate the fumigations. With liquid decontamination technologies, neutralization of the technology occurred at the end of the contact time, at the same time as the extraction of the coupon. Neutralization of liquid decontamination technologies is described in the section reporting specific liquid decontamination technologies. To extract the biological agent, the coupons were placed individually into sterile 50 mL conical vials to which 10.0 mL of sterile extraction buffer was added. Phosphate-buffered saline was the extraction buffer for ricin and vaccinia virus. Phosphate-buffered saline with 0.1% Triton X-100 (Sigma) was the extraction buffer for spores.[2] The tubes were agitated on an orbital shaker for 15 min at approximately 200 rpm at room temperature.

2.6.1 B. anthracis Spores

The number of residual viable *B. anthracis* spores on test and positive control coupons was determined using a dilution plating approach on tryptic soy agar (TSA). *B. anthracis* grows well on ordinary laboratory media.[1] Following extraction, the extract was removed and a series of dilutions through a maximum of 10^{-7} were prepared in sterile water. An aliquot (0.1 mL) of the undiluted extract and each serial dilution were plated onto TSA plates, shown in Figure 2-6, in triplicate. The cultures were incubated for 18-24 hr at 37 °C ± 2 °C. Colonies (CFU) were counted manually. The number of CFU/mL was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data were expressed as mean CFU/mL ± SD based on the numbers of CFU observed.

Potential confounding organisms were excluded or controlled by sterilization of the coupons (by autoclaving or gamma irradiation), use of sterile technique, and a pure initial culture. Negative control coupons were run in parallel with the inoculated coupons (both test and positive control coupons). Except as noted in Section 3.5.3, no CFU were observed on any blanks so the CFU observed from inoculated coupons were assumed to indicate viable *B. anthracis* spores and were confirmed by observation of colony morphology.

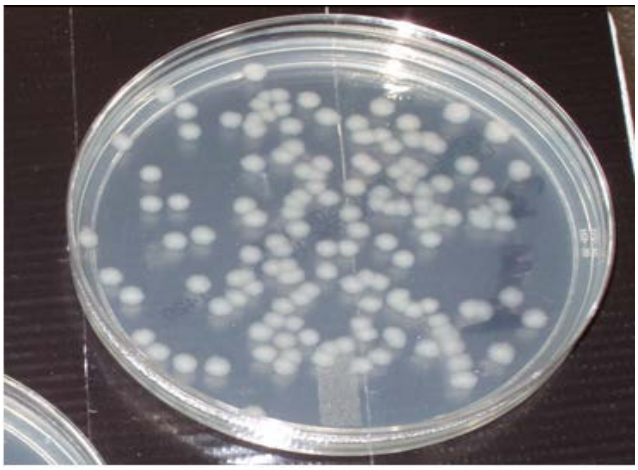


Figure 2-6. *B. anthracis* Ames colonies on TSA.

2.6.2 Ricin

The amount of cytotoxic ricin persisting on test coupons over time was determined using a bioassay. The bioassay used in this investigation for determining the cytotoxicity of bioactive ricin was based on the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5,-diphenyltetrazolium bromide) assay developed by Mosmann (1983).[4] The mechanism of action by which ricin exerts its toxic effect is through inhibition of protein synthesis within cells. Such inhibition of protein production leads to cell death. Therefore, an *in vitro* cytotoxicity assay can be used to evaluate the cytotoxicity of bioactive ricin in the applied volume of solution. Cytotoxicity was reported as remaining mass of bioactive ricin relative to initial cytotoxic protein mass applied.

Vero (African Green Monkey kidney) cells were seeded in wells of a 96-well microplate at a density of approximately 2×10^4 cells/well. Cells were cultured to confluence (approximately 16-30 hr) at 37 °C ± 2 °C under 95% air and 5% CO_2 and exposed to the coupon extracts. Following extraction of coupons, 1.0 mL of the PBS extracts of the ricin test and control coupons were removed and an 8-point series of two-fold dilutions was prepared in complete cell culture medium. An aliquot (100 µL) of the undiluted extract and each serial dilution were plated onto confluent Vero cells in the 96-well plate. For quantitation, a parallel standard curve was prepared using 2- or 3-fold serial dilutions of 10 ng/mL ricin and plated onto confluent Vero cells in the 96-well plate. Following exposure to coupon extracts for 72 hr at 37 °C ± 2 °C in a humidified 95% air 5% CO_2 atmosphere, the cells were incubated in the presence of MTT (Promega, Madison, WI) for 4 hr, where mitochondrial enzymes within cells convert the yellow MTT to a purple formazan salt. The reaction was terminated by adding 100 µL of a Solubilization/Stop Solution (Promega, Madison, WI) and incubation at 37 °C ± 2 °C in a humidified atmosphere for 1-24 hr. A SPECTRAmax microplate reader (Molecular Devices, Silicon Valley, CA) was used to measure the absorbance at 570 nm wavelength using a reference wavelength of 630-750 nm. For each standard and test sample, absorbance values of the reference wavelength (630-750 nm) were

subtracted from the absorbance values at 570 nm for each well. Absorbance values were directly proportional to the number of viable cells present in the sample well. For each standard, the mean absorbance values (Y-axis) were plotted against the concentration in ng/mL, and a four-parameter logistic curve was generated by the software included in the SPECTRAmax microplate reader using Equation 1

$$Y = \min + \frac{(\max - \min)}{1 + (X / C)^{B}} \qquad \text{Equation 1}$$

where:

X = concentration of ricin, ng/mL
max = Y-value of the asymptote at the low values of X, optical density
min = Y-value of the asymptote at the high values of X, optical density
B = value related to the slope of the curve between the asymptotes
C = X-value of the midpoint between max and min, ng/mL.

The absorbance of the purple reaction product, read at 570 nm using a microplate reader, was directly proportional to the number of living cells. The amount of purple formazan produced was inversely proportional to the cytotoxic potential of ricin (Figures 2-7 and 2-8).

To determine the concentration of ricin toxin from each test sample (i.e., the mass of ricin toxin extracted in a specified volume of extraction fluid), the ricin toxin stock solution (as received from Vector Laboratories) was assayed in parallel and used to prepare a standard curve of absorbance vs. mass of ricin protein. The absorbance of sample extracts was plotted onto the standard curve to determine the mass of cytotoxic ricin in the sample. Data were expressed as the concentration of bioactive ricin. The percent cytotoxic ricin recovery was calculated.

In the method development, the inherent cytotoxicity of extracts of the various types of coupons was determined. The coupons with background levels of cytotoxicity were diluted sufficiently that the cytotoxicity of the extract (without ricin) killed less than 10% of the cells in the MTT assay. The dilution scheme effectively "zeros out" the cytotoxicity of the test coupons (see Tables 2-2).

Table 2- 2. Dilution Factors for Various Coupon Materials

Material	Dilution Factors Required to "Zero Out" Coupon Cytotoxicity*
Glass	1:4
Painted Concrete	1:4
Galvanized Metal	1:4
Decorative Laminate	1:4
Cellulose Insulation	1:256
Particle Board	1:32
Industrial Carpet	1:64

*Coupon cytotoxicity is considered to be "zero-ed out" if the optical density readings after cell exposure to coupon extracts are at least 90% of media alone.

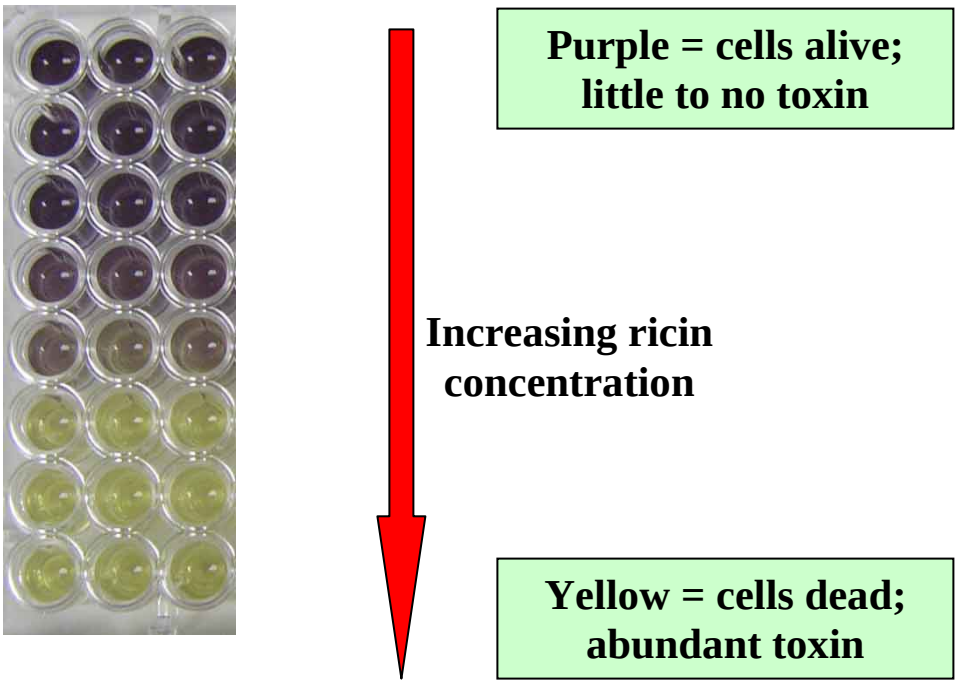


Figure 2-7. Visual demonstration of MTT assay on a microplate.

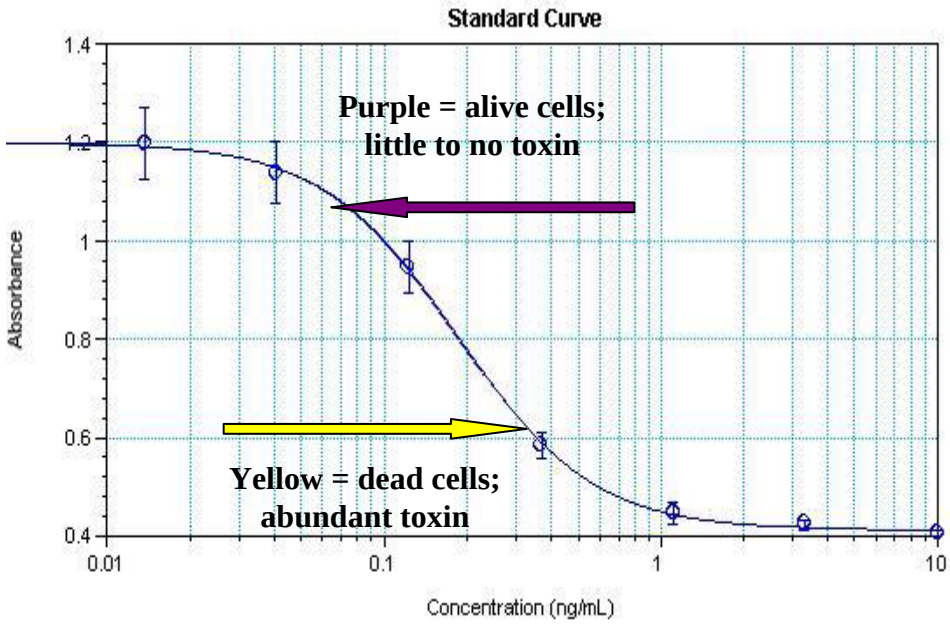


Figure 2-8. Example of ricin cytotoxic profile with corresponding absorbance measured using microplate reader.

2.6.3 Vaccinia Virus

Both vaccinia and *variola* (smallpox) viruses are species in the genus *Orthopoxvirus*. There is a 96% identity at the nucleotide level between vaccinia and *variola*.[5] Given the high levels of similarity between these viruses, decontamination of *variola* is expected to be similar to that observed for vaccinia.[6] The investigation of the decontamination efficacy against vaccinia virus measured the amount of residual PFU extracted from test and control coupons. The number of vaccinia viruses on the coupons was determined using a dilution plating approach. Following extraction, 1.0 mL of the PBS extract was removed and a series of dilutions through 10^{-7} was prepared in sterile water or PBS. An aliquot (100 µL) of the undiluted extract and each serial dilution were plated onto uninfected Vero cells. Following inoculation, the tissue culture plates were rocked for approximately 1 hr at 37 °C ± 2 °C to allow adsorption of vaccinia virus to the Vero cells. The cultures were then overlaid with Minimum Essential Medium containing 2% fetal bovine serum, 0.5% methylcellulose, and antibiotics. The cultures were incubated for 1-2 days at 37 °C ± 2 °C in 95% air and 5% CO_2. Following incubation, crystal violet dye was added to the monolayers for 15 min, removed and the cells rinsed with PBS. Plaques were visualized as clearings in the purple monolayer of Vero cells and the PFU were counted manually. The number of PFU/mL was determined by multiplying the average number of plaques per well by the reciprocal of the dilution. Data were expressed as mean PFU/mL ± SD based on the numbers of PFU observed.

Potential confounding organisms were excluded or controlled by sterilization of the coupons, use of sterile technique, negative control coupons and a pure initial culture. Negative control coupons were run in parallel with the inoculated coupons. No PFU were observed on any blank control coupons. Therefore, the PFU observed from inoculated coupons were assumed to indicate viable vaccinia virus.

2.7 Broth Culture Assay for Viable Spores (Qualitative Method)

B. anthracis grows in ordinary laboratory media,[1] including nutrient broth (see, for example CDC, American Society for Microbiology, and Association of Public Health Laboratories[7]), at 37 °C. A qualitative broth culture assay was used to detect the presence of viable spores on the BIs or residual on coupons after extraction. The BIs or coupons were aseptically transferred into individual tubes containing 30 mL of tryptic soy broth (TSB) culture medium (as specified in the respective test/QA plans) and capped. The tubes were cultured for seven days at 37 °C ± 2 °C to encourage viable spore germination and subsequent proliferation of vegetative bacteria.

At one and seven days post-decontamination, the tubes were visually assessed for cloudiness. A cloudy culture medium may indicate “growth” of viable spores. Clear culture medium indicated “no growth” and was consistent with a complete kill of all spores on the BI or complete kill or extraction of all viable

spores on/from the coupon. Data were expressed as "growth" ("+") or "no growth" ("-").

In an approach, referred to herein as qualitative cycle fractionation, coupons are removed from test conditions at various contact times during the decontamination cycle, placed into TSB, and treated as described in the preceding paragraphs. Data are reported as "growth" or "no growth" after a given contact time. This approach was used selectively as a screening procedure to refine or determine test conditions.

2.8 Calculations and Statistics

2.8.1 Percent Recovery

Percent recovery for each biological agent on each test material was determined prior to investigating decontamination efficacy. Percent recovery (mean ± SD) was calculated for each type of test material inoculated with each biological agent by dividing the viable biological agent (spores or vaccinia virus) or mass of cytotoxic ricin extracted from control coupons by the number of biological organisms or ricin mass applied to each coupon.

Percent recovery (% R) is calculated as:

$$\%R = \left(\frac{x}{A}\right) \times 100 \qquad \text{Equation 2}$$

where:

x = CFU/mL, PFU/mL or mass of cytotoxic ricin recovered from the control coupons

A= CFU/mL, PFU/mL or mass of cytotoxic ricin applied to the control coupons

The quantity applied was determined during the confirmation of initial titer using analytical methods described in Section 2.6. Mean percent recovery was calculated by summing the replicate percent recovery values and dividing by the number of replicates (five).

2.8.2 Decontamination Efficacy

Decontamination efficacy was calculated as the log reduction (mean ± SD) in viable biological agent (CFU or PFU) or percent reduction in mass of cytotoxic ricin after a given treatment. The higher the decontamination efficacy (log reduction or percent reduction) value, the less biological agent remains on the test coupon after a given treatment.

The first step in the calculation of overall decontamination efficacy for viable biological agents (spores or viruses) was to determine the efficacy for each individual coupon in a given set of replicates. The efficacy was defined as the extent (expressed as log reduction) to which the specific agent extracted from the specific type of coupon after the treatment with the decontamination technology at a specific concentration for a specific contact time (at a given temperature and RH) was less than what was extracted from coupons exposed to the contact time, temperature, and RH portions of the treatment without exposure to fumigation. Efficacy of a given treatment against a specific viable biological agent on an individual coupon was calculated in the general form:

$$Efficacy_k = \frac{\sum_i \log_{10}(x_i)}{i} - \frac{\sum_j \log_{10}(t_j)}{j} \qquad \text{Equation 3}$$

Where:

x = cytotoxicity (µg), CFU or PFU values of an individual control coupon of a given type and condition

t = measured cytotoxicity (µg), CFU, or PFU value of an individual test coupon of a given type and treatment

k = particular coupon type and test condition

i = number of control coupons

j = number of test coupons

A t-test on the efficacy data was used to statistically analyze whether a decontamination treatment resulted in significant efficacy. The t-test was performed using PROC TTEST in SAS v 9.1. It was assumed that the two underlying populations to be compared were normally distributed. A folded F-test was performed to assess whether they have equivalent variances. If they had equivalent variances, a pooled variance estimate was used. Otherwise, the separate sample variances were used and the Satterthwaite approximation for degrees of freedom was employed. The PROC TTEST generated an estimated mean log reduction, 95% confidence interval, and a p-value for statistical significance. Where the p-value was less than or equal to 0.05, and assuming a positive estimate, it was concluded that the reduction in log recovery due to the treatment was superior to that of the controls alone with 95% or greater confidence. Note that cytotoxicity was transformed back into the original units (micrograms) and is reported as a geometric mean with a 95% confidence interval. The geometric mean has an asymmetric 95% confidence interval.

Model diagnostics were examined to assess whether there were any difficulties with outliers or the model assumptions of constant variance and normality of the residuals. For data that were not adequate for the model, appropriate transformations or more general statistical models (e.g., nonparametric) were used. No outlier was identified and excluded from the final analysis.

To make comparisons between different experimental groups (e.g., CTs, *Bacillus* strains, humidity levels), the approach above was extended to a general linear model with PROC GLM in SAS v 9.1.

If test coupons had zero recovered CFU or PFU, the base 10 logarithm was undefined. In these cases, the value of zero was replaced by 1 CFU or PFU. The arithmetic average log reduction was calculated as above, but the reported value was a conservative reduction, and was identified as such by "> x" log reduction in the results. The t-test assumptions of normality are not valid in these cases, and the statistical comparison cannot be done with a t-test. Instead, a nonparametric approach was taken. The Kolmogorov-Smirnov test

was performed through PROC NPAR1WAY in SAS v 9.1. If a p-value of 0.05 or less was found, the overall distribution of the treatment was concluded to be different than the controls.

If both the control and decontamination coupons have occurrences of zero recovered CFU or PFU, the log reduction becomes indeterminate as defined above. A statistical test of differences using the Kolmogorov-Smirnov procedure was performed with the interpretation as above.

There were separate statistical analyses for *B. anthracis*, *B. subtilis*, vaccinia virus, and ricin. The specific factors included in the analyses vary among technologies and trials.

The efficacy data for biological organisms are reported as log reductions. Efficacy data for ricin toxin are reported as percent reductions. The primary decontamination efficacy results from the coupon testing are shown in a matrix table in which each entry shows the "geometric mean log reduction" in viable organisms or "mean percent reduction" in ricin cytotoxicity for each combination of biological agents, surface materials, concentration, contact time, temperature, and RH.

Statistical analysis in this section compares whether the efficacy of the decontamination treatment at a particular concentration, contact time, temperature, RH, and test material was significantly ($p \leq 0.05$) different from zero (null hypothesis). Additional comparisons were made of mean efficacy between materials, contact time, temperature, and RH. Both means and corresponding p-values were produced for each comparison.

2.9 ClO_2 Fumigation

2.9.1 Description of ClO_2 Technology

The Sabre decontamination technology in this investigation uses ClO_2 as the active ingredient for decontamination. ClO_2 is not stable as a compressed gas and, therefore, ClO_2 gas must be produced on-site. For this evaluation, Sabre Technical Services, LLC, provided chemicals and a custom-built bench-scale system for on-site generation, delivery, removal, and neutralization of ClO_2. The decontamination technology was operated as specified an the standard operating procedure developed with the vendor and summarized below.

The Sabre equipment includes a 20 cm base onto which was mounted a 15 cm square, 91 cm high sparging column. An aqueous ClO_2 solution consisting of about 3 g/L of ClO_2 plus 1000 ppm of chlorite for a 3000 ppm ClO_2 solution was prepared on-site; however, these values varied slightly from batch to batch. Because the ClO_2 is sparged into the test chamber atmosphere, the exact concentration of the aqueous solution is not critical. ClO_2 solution was pumped (using a peristaltic pump) into the sparging column and air from the test chamber was pumped into and through the column to sparge the ClO_2 from the liquid into the air stream. The air stream re-entered the test chamber to establish the desired gaseous ClO_2 concentration. Liquid introduction from the reservoir of ClO_2/chlorite solution to the sparging column was initially at the rate of 60 mL per min; when the desired ClO_2

concentration in the test chamber was achieved, the liquid introduction into the sparging column was turned off. As the ClO_2 concentration dropped, additional gas was added to the chamber by manually turning on the flow of air through the sparging column and back into the chamber to achieve the target concentration. The spent liquid exiting the sparging column was collected in a reservoir. The air from the test chamber was recirculated into and out of the sparging column.

2.9.2 ClO_2 Test Matrix

The testing performed in this investigation is shown in Table 2-3. Critical parameters include ClO_2 concentration, decontamination contact time, temperature, RH, and the viability or cytotoxicity of the biological agents. Efficacy of ClO_2 is generally assumed to be higher at higher concentrations, with longer contact times, at an RH of 75% or higher, and at an elevated temperature. These assumptions were tested. An adaptive management approach was used to incorporate new knowledge into the testing. Based on new knowledge, EPA revised, added or eliminated trials and experimental conditions. These changes were documented in amendments to the original test/QA plan.

The trial numbers correspond to trials specified in the test/QA plan and amendments. The trial numbers are included for convenience in referencing back to the test/QA plan and amendments for details. Numbering gaps represent deletions by EPA of planned trials. Numbers with a letter (e.g., 4a) represent trials that were added by amendment of the test/QA plan. Results from all testing under the test/QA plan, including testing done under amendments, are included in this report.

Five replicate test coupons (plus one procedural blank) and five replicate positive control coupons (plus one laboratory blank) were included at each set of conditions and time points except in Trials 4a, 4b, and 4g. Trials 4a, 4b, and 4g were run with three replicate test coupons at each time point, three replicate positive control coupons, a procedural blank, and a laboratory control blank. A smaller number of replicates were used in the qualitative screening tests (three) than in the quantitative tests (five) because no statistical inferences would be drawn. Negative controls (procedural blanks and laboratory blanks) are coupons to which corresponding diluent, but no biological agent, was applied.

Decontamination was halted by dropping the atmospheric concentration of ClO_2 to which the test and procedural blank coupons were exposed to near zero by removal of the coupons from the test chamber into the air inside the BSC III and then into the extraction medium. No quenching agents were added to stop the decontamination reaction because residual ClO_2 was assumed to be rapidly removed from interaction with the biological agent through convection, diffusion, and dilution.

Trials 1-3. The experimental design tested whether there was a difference between the decontamination efficacy using the treatment compared to the control. For any particular material, the comparisons included efficacy under given environmental conditions (temperature and humidity) at a particular CT (concentration of fumigant

x contact time) inactivating a particular biological agent. The dependent variable was extracted residual viable spores measured as CFU, compared to controls. Tests were performed with five replicate test coupons and five replicate positive controls for each set of conditions and time points.

Trials 1 through 3, shown in Table 2-3, used *B. anthracis* (Ames) spores on painted concrete to screen for the impacts of temperature, concentration, and decontamination contact time on log reduction in viable spores. The target ClO_2 concentrations ([ClO_2]) were 3,000 ppmv, 1,500 ppmv, or 750 ppmv, respectively. The target RH was >75%. T0, for all trials, was the time that the ClO_2 reached the target concentration. The target temperature was 24 °C.

Trial 4, shown in Table 2-3, was run at the shortest contact time from Trial 1 with 100% kill (no CFU recovered from any coupon type). The target 3,000 ppmv ClO_2 fumigation was used to decontaminate *B. anthracis* (Ames) spores from eight materials. This concentration was selected for application against a broad range of materials because 3,000 ppmv ClO_2 is the typical application in the field. The target temperature was 24 °C.

Table 2-3. Test Matrix for ClO_2 Decontamination Investigation

Trial	Agent	Material	CT, ppmv-Hr	Contact Time, Hr	Target [ClO_2] ppmv	Target Temperature	Target RH	Number of Coupons per Material Type and Condition				
								Test Coupons	Procedural Blank	BI	Positive Control‡	Lab Blank
1	*B. anthracis* Ames	Painted concrete	**2,250**	0.75	**3000**	24 ± 2 °C	>75%	5	1	1	5	1
1	*B. anthracis* Ames	Painted concrete	**4,500**	1.5	**3000**	24 ± 2 °C	>75%		1	1	5	1
1	*B. anthracis* Ames	Painted concrete	**9000**	3	**3000**	24 ± 2 °C	>75%		1	1	5	1
2	*B. anthracis* Ames	Painted concrete	**2,250**	1.5	**1500**	24 ± 2 °C	>75%	5	1	1	5	1
2	*B. anthracis* Ames	Painted concrete	**4,500**	3	**1500**	24 ± 2 °C	>75%		1	1	5	1
2	*B. anthracis* Ames	Painted concrete	**9000**	6	**1500**	24 ± 2 °C	>75%		1	1	5	1
3	*B. anthracis* Ames	Painted concrete	**2,250**	3	**750**	24 ± 2 °C	>75%	5	1	1	5	1
3	*B. anthracis* Ames	Painted concrete	**4,500**	6	**750**	24 ± 2 °C	>75%		1	1	5	1
3	*B. anthracis* Ames	Painted concrete	**9000**	12	**750**	24 ± 2 °C	>75%		1	1	5	1
4	*B. anthracis* Ames	Eight materials*	**9000**	3	**3000**	24 ± 2 °C	>75%	5	1	1	5	1

* Eight test materials are small glass, painted concrete, galvanized metal, decorative laminate, cellulose insulation, particle board, industrial carpet, large glass.
†Seven test materials are small glass, painted concrete, galvanized metal ductwork, decorative laminate, cellulose insulation, particle board, and industrial carpet.
‡Five positive control coupons extracted at time 0 (the time at which decontamination treatment begins) and five positive control coupons extracted at the end of the treatment time.
Bold: factor varied in trial series
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 2-3. Continued

Trial	Agent	Material	CT, ppmv-Hr	Contact Time, Hr	Target [ClO_2] ppmv	Target Temperature	Target RH	Number of Coupons per Material Type and Condition				
								Test Coupons	Procedural Blank	BI	Positive Control	Lab Blank
5	*B. anthracis* Ames	Seven materials†	9000	3	3000	**30 °C ± 2 °C**	>75%	5	1	1	5	1
6	*B. anthracis* Ames	Seven materials† except cellulose insulation	1000	0.33	3000	**24 °C ± 2 °C**	>75%	5	1	1	5	1
6	*B. anthracis* Ames	Cellulose insulation	12,000	4	3000	24 °C ± 2 °C	>75%	5	1	1	5	1
9	Ricin	Eight materials*	500	0.33	**1500**	24 °C ± 2 °C	>75%	5	1	1	10‡	1
10	Ricin	Eight materials*	100	0.5	**200**	24 °C ± 2 °C	>75%	5	1	1	10‡	1
13	Vaccinia	Eight materials*	500	0.33	**1500**	24 °C ± 2 °C	>75%	5	1	1	10‡	1
14	Vaccinia	Eight materials*	125	0.5	**250**	24 °C ± 2 °C	>75%	5	1	1	10‡	1

* Eight test materials are small glass, painted concrete, galvanized metal, decorative laminate, cellulose insulation, particle board, industrial carpet, large glass.
†Seven test materials are small glass, painted concrete, galvanized metal ductwork, decorative laminate, cellulose insulation, particle board, and industrial carpet.
‡Five positive control coupons extracted at time 0 (the time at which decontamination treatment begins) and five positive control coupons extracted at the end of the treatment time.

Table 2-3. Continued

Trial	Agent	Material	CT, ppmv-Hr	Contact Time, Hr	Target [ClO_2] ppmv	Target Temperature	Target RH	Number of Coupons per Material Type and Condition			
								Test Coupons	Procedural Blank	Positive Control‡	Lab Blank
4c	***B. anthracis*** **Ames & *B. atrophaeus* BIs**	ECBC 3.1 x 3.1 cm coupons of ceiling tile, unpainted pine wood, and painted I-beam steel	3000	1	3000	24 ± 2 °C	>75%	5	1	10	1
4d	***B. anthracis*** **Ames with 0.5% bovine serum albumin & *B. atrophaeus* BIs**	ECBC 3.1 x 3.1 cm coupons of ceiling tile, unpainted pine wood, and painted I-beam steel	3000	1	3000	24 ± 2 °C	>75% (83% actual)	5	1	10	1
4e	***B. anthracis*** **Ames with 0.5% bovine serum albumin (ceiling tilie only), *B. anthracis* NNR1Δ1 with 0.5% bovine serum albumin, & *B. atrophaeus* BIs**	ECBC 3.1 x 3.1 cm coupons of ceiling tile, unpainted pine wood, and painted I-beam steel	3000	1	3000	24 ± 2 °C	**75%**	5	1	10	1

* Eight test materials are small glass, painted concrete, galvanized metal, decorative laminate, cellulose insulation, particle board, industrial carpet, large glass.
†Seven test materials are small glass, painted concrete, galvanized metal ductwork, decorative laminate, cellulose insulation, particle board, and industrial carpet.
‡Five positive control coupons extracted at time 0 (the time at which decontamination treatment begins) and five positive control coupons extracted at the end of the treatment time.

Table 2-2. Continued

Trial	Agent	Material	Target $[ClO_2]$ ppmv	Target Temperature	Target RH	Decontamination Contact Times and Number of Coupons of Each Material
4a	*B. anthracis* Ames	Painted concrete, galvanized metal ductwork, decorative laminate, cellulose insulation, particle board, and plate glass.	**750**	24 ± 2 °C	>75%	Three replicates of each material at 0, 10, 20, 30, 40, 50, 60 min are placed into TSB to test for viable spores. (Qualitative test)
4b	*B. anthracis* Ames	Painted concrete, galvanized metal ductwork, decorative laminate, cellulose insulation, and plate glass.	**3000**	24 ± 2 °C	>75%	Three replicates of each material at 0, 20, 40, 60, 80, 100, 120 min are placed into TSB to test for viable spores. (Qualitative test)
4g	*B. anthracis Ames*	Plate glass	3000	24 °C ± 2 °C	70%-75%	Three replicates of each material/ spore combination at 0, 30, 60, 90, and 120 min are placed into TSB to test for viable spores. (Qualitative test)
4g	*B. subtilis*	**Spores on stainless disk in Tyvek® envelope**	3000	24 °C ± 2 °C	70%-75%	Three replicates of each material/ spore combination at 0, 30, 60, 90, and 120 min are placed into TSB to test for viable spores. (Qualitative test)
4g	*B. subtilis*	**Tyvek® envelope, spores on stainless disk removed from Tyvek® envelope)**	3000	24 °C ± 2 °C	70%-75%	Three replicates of each material/ spore combination at 0, 30, 60, 90, and 120 min are placed into TSB to test for viable spores. (Qualitative test)
4g	*B. atrophaeus*	**Spore on filter paper strips in glassine envelope**	3000	24 °C ± 2 °C	70%-75%	Three replicates of each material/ spore combination at 0, 30, 60, 90, and 120 min are placed into TSB to test for viable spores. (Qualitative test)
4g	*B. atrophaeus*	**Spore on filter paper strips removed from glassine envelope**	3000	24 °C ± 2 °C	70%-75%	Three replicates of each material/ spore combination at 0, 30, 60, 90, and 120 min are placed into TSB to test for viable spores. (Qualitative test)

* Eight test materials are small glass, painted concrete, galvanized metal, decorative laminate, cellulose insulation, particle board, industrial carpet, large glass.
†Seven test materials are small glass, painted concrete, galvanized metal ductwork, decorative laminate, cellulose insulation, particle board, and industrial carpet.
‡Five positive control coupons extracted at time 0 (the time at which decontamination treatment begins) and five positive control coupons extracted at the end of the treatment time.

Trials 4a-4b. In order to determine the CT at which zero growth was observed in a qualitative spore viability test, cycle fractionation tests (Table 2-3) were performed. In a cycle fractionation test, the maximum fumigation contact time (decontamination cycle) was divided into a series of shorter contact times by administering a given concentration of fumigant and terminating the exposure time at various intervals up to the full CT being evaluated. The presence of viable spores was evaluated by qualitative analysis (described in Section 2.7).

Indoor building material coupons were spiked with approximately 1 x 10^8 *B. anthracis* Ames spores. The spiked coupons were exposed to a known concentration of ClO_2 (750 ppmv or 3000 ppmv) at a target RH of >75% and a target temperature of 24 °C ± 2 °C. At specified time intervals within the decontamination cycle, three spiked coupons of each material type were removed from the test conditions and placed into nutrient broth and incubated at 37 °C up to 7 days to perform a qualitative assay for viable spores (described in Section 2.7). The time interval for removal of spiked coupons was 10 or 20 min over a total period of 60 or 120 min for Trials 4a and 4b, respectively.

Trials 4c-4e. The EPA was investigating the effectiveness of ClO_2 gas for the inactivation of *Bacillus* species spores in conjunction with ECBC and at Battelle. The preliminary results from the two organizations were shared and appeared to be inconsistent. These trials were added to the original test matrix so that Battelle could replicate the ECBC testing to determine the causes of differences between ClO_2 efficacy results obtained at Battelle and those obtained at ECBC.

Three types of indoor building material coupons previously used in testing at ECBC were provided to Battelle by ECBC for use in trials 4c, 4d and 4e. The coupons, measuring 3.1 cm x 3.1 cm, include ceiling tile, unpainted pine wood, and painted I-beam steel.

In Trial 4c, *B. anthracis* Ames spores (preparation used at Battelle without added organic burden) were spiked onto material coupons used in the EPA/ECBC decontamination testing. In Trial 4c, 15 coupons of each material type were spiked with seven 7.1-µL aliquots (49.7 µL total) of a stock suspension of known concentration of *B. anthracis* Ames (CFU/mL), prepared as described in the test/QA plan. The spiked coupons were dried overnight and then placed into the glove box, described in Section 2.2.1. The coupons were exposed to a known concentration of ClO_2 (3000 ppmv) for a specified contact time (1 hr or 3 hr). After specified contact time intervals, five spiked coupons of each material type were removed from the test conditions, extracted, and enumerated as described in Section 2.6.1. One blank control and five positive control coupons were run at T0 and in parallel with the test coupons.

In the tests at ECBC, organic burden was added to the spore suspension (0.5% bovine serum albumin); Battelle testing does not typically add organic burden to the spore suspension. Battelle performed Trial 4d to evaluate the impact on efficacy of adding organic burden when compared to Trial 4c without organic burden. In Trial 4d, organic burden

(0.5% bovine serum albumin) was added to the *B. anthracis* Ames spores (preparation used at Battelle), and the spores were spiked onto EPA/ECBC material coupons for use in decontamination testing. This procedure provided a comparable level of organic burden to the EPA/ECBC spore preparations and the spores were applied to EPA/ECBC material coupons.

In Trial 4d, fifteen coupons of each material type were spiked with seven 7.1-µL aliquots (49.7-µL total) of a stock suspension of known concentration of *B. anthracis* Ames, prepared as described in the test/QA plan but with 0.5% bovine serum albumin as added organic burden. The spiked coupons were dried overnight and then placed into the glove box, described in Section 2.2.1. The coupons were exposed to a target concentration of ClO_2 (3000 ppmv) at an RH >75% and a temperature of 24 °C ± 2 °C for 1 hr. After the 1-hr contact time, five spiked coupons of each material type were removed from the test conditions, extracted, and enumerated as described in the test/QA plan. One blank control and five positive control coupons were run at T0 and in parallel with the test coupons.

EPA/ECBC uses *B. anthracis* NNR1Δ1 spores with organic burden for some efficacy testing; testing is performed at a 75% RH. In Trial 4e, Trial 4d was replicated, but using the *B. anthracis* NNR1Δ1 with 0.5% bovine serum albumin provided by ECBC and *B. anthracis* Ames with 0.5% bovine serum albumin. Further, the RH was controlled at about 75%, a lower RH than used in previous testing (Trials 1-4b) in the studies conducted at Battelle, but consistent with testing performed at ECBC. The target RH was selected based on the results of Trial 4d. The actual RH in Trial 4d was 83% and condensation was visible on the test chamber. The lower RH in Trial 4e was selected to avoid visible condensation. Trials 4d and 4e compared the "Battelle conditions" of *B. anthracis* Ames at high RH with visible condensation to the "ECBC conditions" of *B. anthracis* NNR1Δ1 with 0.5% bovine serum albumin at 75% RH (no visible condensation). In addition, 5 BIs (*B. atrophaeus* on stainless steel in a Tyvek® pouch (Apex Laboratories)) were included at T0 and at the one-hr time point.

Trial 4g. In Trial 4g, qualitative testing determined the CT at which no viable spores were detected on various materials when the RH was controlled in a target range of 70%-75%, a lower RH than was used in previous trials (>75%). At this lower RH, no liquid water was present in the test chamber. At higher RH conditions of previous trials, moisture and condensation were sometimes observed in the test chamber. The decontamination cycle fractionation exposed spores to a given concentration of fumigant and for exposure time at various intervals up to the full CT being evaluated. The presence of viable spores on coupons after a given CT exposure was determined by incubating the coupons in nutrient broth and checking for cloudiness that would indicate growth from viable spores.

Plate glass coupons (three per time point) were each spiked with a 100 µL aliquot of a stock suspension of known concentration of *B. anthracis* Ames. The spiked coupons were placed into the test chamber along with the following BIs:

- *Bacillus atrophaeus* spores on filter paper strips in glassine envelopes (Raven Biological Laboratories)
- *Bacillus atrophaeus* spore strips on filter paper strips removed from glassine (Raven Biological Laboratories)
- *Bacillus subtilis* on stainless disk in Tyvek® pouches (Apex Laboratories)
- *Bacillus subtilis* on stainless disk removed from Tyvek® pouches (Apex Laboratories).

The coupons were exposed to 3000 ppmv ClO_2 in Trial 4g. The target temperature was 24 °C ± 2 °C, and the RH was controlled between 70% and 75% for the total decontamination cycle. After reaching the target RH but prior to introducing the test coupons into the test chamber, strips of 3M Water Contact Indicator Tape (3M, St. Paul, MN) were placed into the test chamber for 15 min. The indicator tape was removed and examined to determine whether moisture (liquid water) was present. The results of the indicator tape were documented.

B. anthracis exposures were accomplished by having the spiked coupons in closed vials in the test chamber and using the gloves in the glove box to open them in sequence so that an appropriate contact time for exposure to ClO_2 was achieved. Contact time was the time from opening the vial containing the spiked coupon in the fumigation test chamber until the coupon was removed from the test chamber. Time zero coupons were in sealed vials in the test chamber for the full fumigation cycle and removed from the chamber without opening the vials; there was no exposure of the T0 coupons to the fumigant.

Trial 5. The experimental design tested decontamination efficacy by determining whether there was a difference between the log reduction in viable *B. anthracis* Ames spores after fumigation compared to positive controls. Efficacy was tested for spores on a variety of types of indoor building materials. These trials also assessed whether there was any difference in efficacy at a target temperature of 30 °C ± 2 °C rather than 24 °C ± 2 °C (used in Trial 4) during decontamination. The target RH was 75%-85%. The fumigation was 3000 ppmv for 3-hr (CT of 9000 ppmv-hr).

Trial 6. The experimental design tested decontamination efficacy at alternative contact times. Efficacy was tested for spores on a variety of types of indoor building materials. These trials also assessed efficacy at a target temperature of 24 °C ± 2 °C during decontamination, target RH was >75% (desirable range of 75% -85%). The fumigation was 3000 ppmv for 20 min (0.33-hr, CT of 1000 ppmv-hr) for all materials except cellulose insulation. Cellulose insulation was fumigated for 4 hr.

Trials 9 and 10. The experimental design tested whether there was a difference between the decontamination efficacy against ricin toxin using the treatment compared to the positive controls. Efficacy was tested for ricin on a variety of types of indoor building materials. The temperature was 24 °C ± 2 °C and the RH was controlled at 80% ± 5%. These trials were run at two CTs: 500 ppmv-hr (Trial 9) and 100 ppmv-hr (Trial 10).

Trials 13 and 14. The experimental design tested whether there was a difference between the decontamination efficacy against vaccinia virus using the treatment compared to the positive controls for each coupon material. Testing was performed using coupons of a variety of types of indoor building materials. The temperature was 24 °C ± 2 °C and the RH was controlled at 80% ± 5%. These trials were run at two CTs: 500 ppmv-hr (Trial 13) and 125 ppmv-hr (Trial 14).

2.10 MeBr Fumigation

2.10.1 Description of MeBr Technology

MeBr has been registered by EPA for soil fumigation (injected into the soil before a crop was planted to effectively sterilize the soil), commodity treatment (used for post-harvest pest control), structural pest control (used to fumigate buildings for termites, and warehouses and food processing facilities for insects and rodents), and quarantine uses (used to treat imported commodities). Target MeBr fumigant concentrations ([MeBr]) and contact times vary with the commodity or structure being treated, the target pest, and temperature. MeBr is an effective pesticide because it acts as a methylating agent that disrupts an organism's internal chemical reactions. However, the use of MeBr has been phased out by EPA under the Clean Air Act due to its being recognized as an ozone depleting substance. Use, now, requires an exemption by the EPA under appropriate provisions in the Clean Air Act (http://www.epa.gov/ozone/mbr/). Research on the use of MeBr to inactivate *Bacillus* species includes:

- Kolb and Schneiter (1950)[8] used spores of six virulent cultures of *B. anthracis*, which were identified only as being strains of canine, bovine, human, and ovine origin. Tests were conducted at room temperature with MeBr concentrations of 3,400 to 3,900 mg/L for 1 to 72 hr; all moist spores (filter paper inoculated with spore suspensions immediately before exposure) were inactivated after 24 hr-72 hr exposures, whereas some dry spores (filter paper inoculated with spores and dried at 65 ^{o}C-75 ^{o}C for 3 to 4 hr prior to exposure) survived. The study did not specify the strains of *B. anthracis* used, specify RH conditions, use building materials used in this investigation, evaluate the effects of temperature, or calculate decontamination efficacy by log reduction.
- Schade and King (1977)[9] conducted decontamination testing with MeBr and spores of *B. megaterium* B-938 and *B. subtilis* var. niger (recently reclassified as *B. atrophaeus*[10]) applied to filter paper. Only the highest MeBr concentration tested (64 mg/L) at the highest temperature tested (35 °C) showed appreciable activity against the spores (the exposure period was 18 hr and the RH was 32%). The test results indicated that high temperature (e.g., 35 °C) and moderately low RH (e.g., 20% RH) were favorable for the sporicidal activity of MeBr. The study results were provided as number of surviving spores on membrane filters

(decontamination efficacy was not presented), testing was not conducted with *B. anthracis*, and the test did not use building materials used in this investigation.

- Weinberg et al. (2004)[11] reported that a MeBr minimum effective dose of 80 mg/L was lethal to 10^7 spores of *B. anthracis* (specifically, nine different strains: ATCC 10, ATCC 937, ATCC 4728, ATCC 11966, AMES-RIID, ANR-1, Sterne, ATCC 14187, AMES-1-RIID) on glass slides after a 48 hr exposure at 37 °C. Similar testing with *B. atrophaeus* and *B. thuringiensis* showed that these bacteria were more resistant to MeBr with minimum effective doses of >112 mg/L. Additional fumigation testing with a MeBr concentration of 120 mg/L at 27 °C resulted in complete mortality of two strains and reductions of seven others. The efficacy of MeBr was reduced at the lower temperature, even when spores were exposed to a 50% higher MeBr concentration. Although RH was monitored during the test, the RH level during decontamination was not reported.
- Weinberg and Scheffrahn (2004)[12] conducted a field trial within a 30,000 cubic foot structure. Filter paper coupons containing 10^6 spores of one of three species, *Geobacillus stearothermophilus*, *B. atrophaeus* and *B. thuringiensis*, and stainless steel coupons with 10^6 spores of *B. atrophaeus* were placed in 50 locations within the structure. After fumigation with 312 mg/L of MeBr for 48 hr at 35.5 °C (the overall mean RH was 76%), only one location (a sealed refrigerator) contained viable spores of *B. atrophaeus* on a single coupon.

2.10.2 Test Matrix for MeBr Fumigation

The research conducted by Weinberg et al. (2004)[11] and Weinberg and Scheffrahn (2004)[12] provides the most pertinent information for the investigations described in this report, which applied *B. anthracis* Ames to new materials (the Weinberg studies only used glass, filter paper, and stainless steel coupons); used a range of MeBr concentrations above and below the minimum effective dose of 80 mg/L[11]; and further investigated the potential influence of RH. Critical parameters include MeBr concentration, decontamination contact time, temperature, RH, and the viability of *B. anthracis* Ames and surrogate spores. The MeBr test matrix is shown in Table 2-4. The preliminary temperature (36 °C) and RH (75%) selected for the test matrix were based on those demonstrated to be effective by Weinberg and Scheffrahn[12] (temperature 35.5 °C and 76% RH). Lower temperatures (25 °C) and RH (40%) were also included in the test matrix.

The theoretical MeBr concentrations selected for this investigation were 80, 160, and 320 mg/L, consistent with the effective levels applied in previous research summarized above, and at levels that may be sustained within a building. The theoretical mass sufficient to generate MeBr at these concentrations

in the test chamber was introduced to the test chamber and the concentration was measured. The measured concentrations were lower than the theoretical concentrations. Thus, introduction of a mass of MeBr theoretically sufficient to generate a concentration of 80 mg/L theoretical mass yielded a measured concentration of about 53 mg/L when measured; 160 mg/L theoretical was about 105 mg/L when measured; and 360 mg/L theoretical was about 212 mg/L when measured. The target measured MeBr concentrations were 53 mg/L, 105 mg/L and 212 mg/L. The results were reported based on measured MeBr concentrations.

Table 2-4. Test Matrix for Generation of MeBr Decontamination CT Curves against *B. anthracis* Ames Spores and Surrogates

Trial	Material	CT, mg/L-Hr	Contact Time, hr	Target [MeBr] mg/L	Target Temperature	Target RH	Number of Coupons per Material Type and Condition				
							Test Coupons	Procedural Blank	BI	Positive Control	Lab Blank
1	Glass, ceiling tile	2520	24	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
2, 13	Glass, ceiling tile	1260	12	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
3	Glass, ceiling tile	315	3	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
4	Glass, ceiling tile	630	6	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
5	Glass, ceiling tile	945	9	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
6	Glass, ceiling tile	1272	6	212 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
7	Glass, ceiling tile	1908	9	212 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
8	Glass, ceiling tile	1484	7	212 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
9	Glass, ceiling tile	1484	7	212 ± 10%	36± 1 °C	40% ± 5% (full scale)	5	1	1	5	1
10	Glass, ceiling tile	795	15	53 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
11	Glass, ceiling tile	954	18	53 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
12	Glass, ceiling tile	1272	24	53 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1

Table 2-4. Continued

Trial	Material	CT, mg/L-Hr	Contact Time, Hr	Target [MeBr] mg/L	Target Temperature	Target RH	Number of Coupons per Material Type and Condition				
							Test Coupons	Procedural Blank	BI	Positive Control	Lab Blank
14	Seven materials*	1260	12	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
15	Seven materials*	1908	9	212 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
16	Seven materials*	1890	18	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
17	Seven materials*	1575	15	105 ± 10%	36± 1 °C	75% ± 5% (full scale)	5	1	1	5	1
18	Eight materials[H]	1908	9	212 ± 10%	25 ± 2 °C	75% ± 5% (full scale)	5	1	1	5	1
19	Eight materials[H]	5088	24	212 ± 10%	25 ± 2 °C	75% ± 5% (full scale)	5	1	1	5	1

*Glass, ceiling tile, cellulose insulation, painted concrete, industrial carpet, galvanized metal ductwork, and decorative laminate. Five silk suture loop carriers prepared per AOAC 966.04 (1×10^4 to 1×10^5) were included in the test chamber during the decontamination; these were evaluated using the qualitative method to test for the presence of viable spores. In addition, *B. subtilis* (prepared per AOAC 966.04 Method 2) on three glass coupons (5 mm x 5 mm) was included in the test chamber during the decontamination; these coupons were evaluated using the quantitative method to enumerate viable spores.

[H]Glass, ceiling tile, cellulose insulation, painted concrete, industrial carpet, galvanized metal ductwork, decorative laminate, pine wood. Five BIs that are *B. atrophaeus* on steel in Tyvek® packaging (Raven).

Note [MeBr] indicates the MeBr concentration in the chamber air, in the units indicated.

The fumigant technology investigated was 99.5% pure MeBr with approximately 0.5% chloropicrin added as a warning odorant. Results were reported as log reduction in viable spores recovered from coupons after a specified contact time and temperature. Five replicate test coupons (plus one procedural blank) and five replicate positive control coupons (plus one laboratory blank) were included at each set of conditions and time points. Positive control coupons and laboratory blanks were run under the same conditions (temperature and RH) and extracted at the same time points as the test coupons. Negative controls (procedural blanks and laboratory blanks) were coupons to which corresponding diluent, but no biological agent, was applied. Because coupons could not be removed from the test chamber while it was charged with MeBr, each contact time constituted a separate fumigation cycle.

The initial concentration of MeBr was established by specifying the concentration, flow rate and time for the injection of MeBr into the test chamber. Theoretical concentrations (based on perfectly stirred reactor [PSR] modeling performed by EPA) are shown Table 2-5. For the MeBr investigation, theoretical MeBr concentrations of 80, 160, and 320 mg/L were selected for the fumigations. The PSR results were used to establish the charging times required to yield these theoretical MeBr concentrations.

Table 2-5. Formula, Model Parameters, and Relevant Model Results

$$V(dC/dt) = Q(C_{in} - C)$$

$Q = 1$ L/min = 0.0167 L/sec
$V = 23$ L
$C_{in} = 3711.8$ mg/L

t [sec]	C [mg/L]
30	79.8
31	82.5
60	157.9
61	160.5
124	319.0
125	321.4

A Fumiscope MeBr meter (Key Chemical & Equipment, Clearwater, FL), of the type used in the field to monitor MeBr concentrations, was used in this investigation to take reading of the MeBr concentration in the test chamber. Fumiscope meter readings were taken after the appropriate charging time for each of the selected theoretical concentrations (80, 160, and 320 mg/L). The charging and Fumiscope meter measurements of MeBr concentration were repeated twice. The means of the initial concentrations, ±10%, were used as the target levels for fumigation in this investigation. For example: Based on the

PSR model, the desired MeBr concentration of 160 mg/L is achieved by introducing 1 standard liter per min of 99.5% MeBr for 61 seconds into a 23 L chamber. At the end of the 61 second injection, the concentration in the chamber was measured using the Fumiscope meter. The initial meter reading was about 105 mg/L. Therefore, the concentration that was maintained in the subsequent investigation, based on the Fumiscope meter reading at 105 mg/L ± 10%, was a Fumiscope meter range of 95 – 116 mg/L for the specified contact time. The test matrix, shown in Table 2-4, shows the target MeBr concentration range based on target Fumiscope meter readings. The target concentrations of MeBr used were 53 mg/L ± 10%, 105 mg/L ± 10%, and 212 mg/L ± 10%.

A comparison, repeated twice, was made between measurements using a total hydrocarbon analyzer (Model M-20, VIG Industries, Anaheim, CA) and the Fumiscope meter. The Fumiscope meter has a positive bias (compared to the total hydrocarbon analyzer) of 10%-20% over the range of interest. Thus, a mean Fumiscope measurement of 53 mg/L corresponded to a mean measurement of 49 mg/L using the total carbon analyzer; a 104.5 mg/L Fumiscope measurement corresponded to a mean measurement of 90.9 mg/L using the total carbon analyzer; and a mean Fumiscope measurement of 211.5 mg/L corresponded to a mean measurement of 179 mg/L using the total carbon analyzer. The MeBr measurements shown in Table 2-4 and in the remainder of this document reflect target or actual MeBr measurements made using the Fumiscope meter during the investigation. Use of Fumiscope-generated data maximizes the utility of the results for field applications in which a Fumiscope meter would likely be used.

The experimental treatments in Trials 1 through 13, shown in Table 2-4, generated data to prepare CT efficacy curves for *B. anthracis* Ames spores on two types of materials at three different concentrations of MeBr. The coupons used were glass and ceiling tile. Except for Trial 9, in Trials 1 through 13, RH was 75% ± 5% (full scale) and temperature was 36 °C ± 1 °C. In Trial 9 the target RH was 40% ± 5% (full scale). Time zero for all trials, was the time that the MeBr reached the target concentration.

The experimental treatments in Trials 14 through 17, also shown in Table 2-3, generated data necessary to prepare CT efficacy curves for MeBr fumigation of *B. anthracis* Ames spores on seven material types. The RH was 75% ± 5% (full scale) and temperature was 36 °C ± 1 °C. Two MeBr concentrations and three contact times were included in the investigation. The seven types of coupon materials used were: glass, painted concrete, galvanized metal, decorative laminate, cellulose insulation, ceiling tile, and industrial carpet. In addition, silk suture loops of the type utilized for efficacy testing in the AOAC 966.04 method[13] were included in qualitative testing.

2.11 HP Fumigation

The STERIS VHP® Generator Series 1000ED (STERIS Corporation, Mentor, OH) was used to introduce hydrogen peroxide (HP) vapor and control the fumigation cycle parameters. Because HP vapor is not stable as a compressed

gas, HP must be produced on site by vaporization of concentrated aqueous solutions of HP. Thus, this technology includes the equipment and chemicals for on-site generation, dispersion, and neutralization of the HP vapor**.**

The HP fumigation technology operates at ambient temperature and atmospheric pressure in a closed loop configuration. As depicted in Figure 2-9 (from STERIS literature), the testing chamber was subjected to four phases: dehumidification, condition, decontamination, and aeration. During dehumidification the RH was reduced by the HP fumigation technology by re-circulating the air through a reusable or disposable desiccant cartridge. Once the desired RH was reached, the automated STERIS VHP® system injected HP vapor at the rate programmed into the system to achieve the desired concentration of HP vapor inside the chamber. The STERIS VHP® system then maintained the set concentration for the desired contact period for decontamination of the biological agent. Once the decontamination phase was completed, the STERIS VHP® system automatically re-circulated the test chamber air through the STERIS VHP® system to reduce the HP vapor concentration to the desired level necessary to safely open the test chamber.

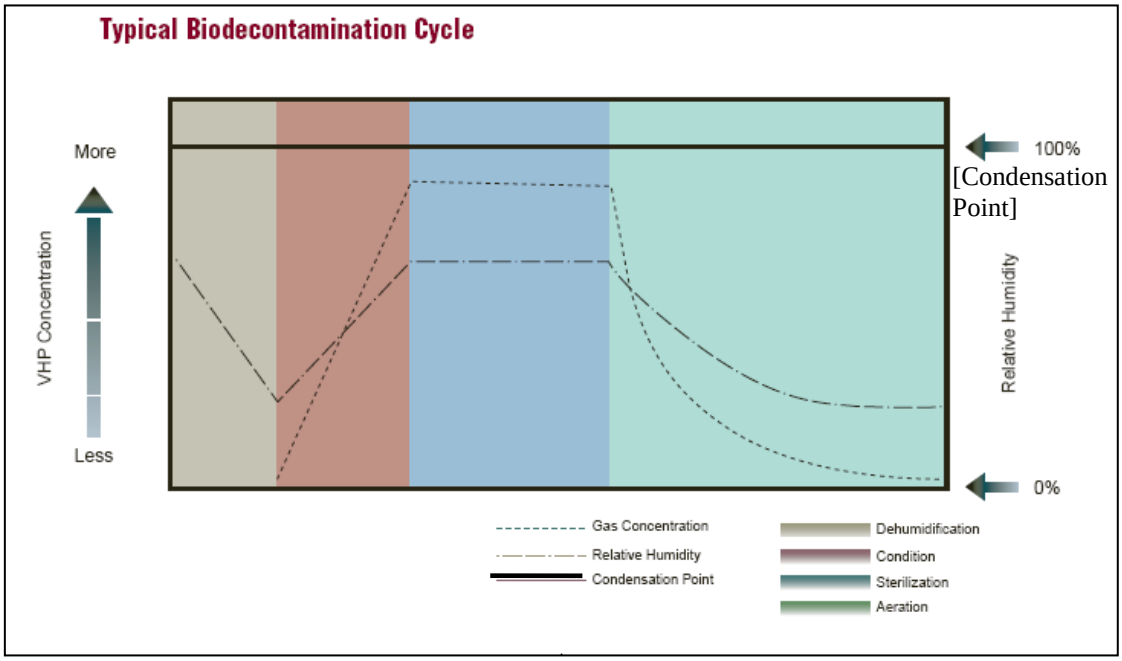


Figure 2-9. STERIS VHP® Biodecontamination Cycle.[14]

The experimental design tested whether there was a difference between the number of viable *B. anthracis* Ames spores recovered from coupons after exposure to the STERIS VHP® fumigation treatment (test coupons) compared to the number of viable spores recovered from positive control coupons that were not exposed to the fumigation treatment. Results are reported as log reduction in viable spores recovered from coupons after a specified contact

time and temperature. Five replicate test coupons (plus one procedural blank) and five replicate positive control coupons (plus one laboratory blank) were included at each set of conditions and time points. Positive control coupons and laboratory blanks were run under the same conditions (temperature and RH) and extracted at the same time points as the test coupons. Negative controls (procedural blanks and laboratory blanks) were coupons to which corresponding diluent, but no biological agent, was applied.

During the test cycle, the spiked coupons were in closed vials in the test chamber. Using the gloves in the glove box the vials were opened in sequence and the coupon removed from each vial so that an appropriate contact time for exposure to HP was achieved. Contact time was the time from opening the vial containing the spiked coupon in the fumigation test chamber until the end of the sterilization cycle. One set of spiked test coupons, along with procedural blank coupons, was not in vials, but was in the test chamber exposed to the full fumigation cycle. Time zero positive control coupons were in sealed vials in the test chamber for the full fumigation cycle and removed from the chamber without opening the vials; there was no exposure of the T0 coupons to the fumigant.

The experimental treatments, shown in Table 2-6, generated data to prepare CT efficacy curves for *B. anthracis* Ames spores on twelve types of materials at four different CTs and a full 4-hr fumigation cycle. The twelve types of coupon materials used were: finished aluminum, computer keyboard keys, industrial grade carpet, painted joint tape, decorative laminate, galvanized metal ductwork, latex-painted concrete, pine wood, plate glass, ceiling tile, particle board, and compressed cellulose insulation. The CTs (concentration x time) that were used for the HP fumigation testing were: 250 ppmv-hr (500 ppmv x 0.5 hr), 500 ppmv-hr (500 ppmv x 1 hr), 1,000 ppmv-hr (500 ppmv x 2 hr), and 2,000 ppmv-hr (500 ppmv x 4 hr).

Table 2-6. Test Matrix for Generation of HP Decontamination CT Curves against *B. anthracis* Ames Spores

Trial	Materials	Target STERIS VHP® Cycle, [HP], ppmv	Type and Number of Coupons or BIs per Contact Time and Material					
			Time 0	Time 1	Time 2	Time 3	Time 4	Time 5
1	Finished aluminum, computer keyboard keys, industrial carpet, and painted joint tape	500	C=3 BL=1	**30 min** N=5 BI=5	**1 hr** N=5 BI=5	**2 hr** N=5 BI=5	**4 hr** N=5 C=2 BI=5	**Gassing + 4 hr** N=5 BI=5
2a	Decorative laminate, galvanized metal ductwork, industrial grade carpet, painted concrete block	500	C=3 BL=1	**30 min** N=5 BI=5	**1 hr** N=5 BI=5	**2 hr** N=5 BI=5	**4 hr** N=5 C=2 BI=5	**Gassing + 4 hr** N=5 BI=5
2b	Industrial grade carpet, decorative laminate, galvanized metal ductwork, painted concrete block	200- 250	C=3 BL=1	**30 min** N=5 BI=5	**1 hr** N=5 BI=5	**2 hr** N=5 BI=5	**4 hr** N=5 C=2 BI=5	**Gassing + 4 hr** N=5 BI=5
3a	Pine wood, plate glass, and ceiling tile	500	C=3 BL=1	**30 min** N=5 BI=5	**1 hr** N=5 BI=5	**2 hr** N=5 BI=5	**4 hr** N=5 C=2 BI=5	**Gassing + 4 hr** N=5 BI=5
3b	Pine wood, plate glass, and ceiling tile	200- 250	C=3 BL=1	**30 min** N=5 BI=5	**1 hr** N=5 BI=5	**2 hr** N=5 BI=5	**4 hr** N=5 C=2 BI=5	**Gassing + 4 hr** N=5 BI=5
D2	Particle board, Cellulose insulation	200- 250	C=3 BL=1	**30 min** **N=5** **BI=5**	**1 hr** **N=5** **BI=5**	**2 hr** **N=5** **BI=5**	4 hr N=5 C=2 BI=5	Gassing + 4 hr N=5 BI=5

C = positive control coupon, BL = laboratory blank, N = test coupon, BI = biological indicator
Note [HP] indicates the HP vapor concentration in the chamber air, in the units indicated.

2.12 Soak in pH-Amended Bleach

The decontamination efficacy of "pH-amended bleach" was investigated. pH-Amended bleach solution was prepared as described in the U.S. EPA crisis exemption requirements for use against *B. anthracis* spores.[15] The solution was prepared using 9.4 parts water, 1 part Clorox® bleach (sodium hypochlorite 5-6%), and 1 part 5% acetic acid to yield a solution having a mean pH close to, but not above, neutral (actual pH = 6.51-6.93) and a mean total chlorine content of 6,000 – 6,700 ppm.

To stop the decontamination action of the pH-amended bleach solution and

extract the biological agent from coupons, the coupons were removed from the decontaminant and placed individually into sterile 50 mL conical vials to which 10.0 mL of sterile extraction buffer with neutralizer was added. Neutralization approaches were tested in an earlier EPA evaluation.[16] Phosphate-buffered saline with 1% sodium thiosulfate was the extraction buffer for vaccinia virus. Phosphate-buffered saline with 1% sodium thiosulfate and 0.1% Triton X-100 (Sigma) was the extraction buffer for spores. The tubes were agitated on an orbital shaker for 15 min at approximately 200 rpm at room temperature.

Qualitative and quantitative test methods were included in the liquid decontamination test matrix outlined in Table 2-7. The qualitative cycle fractionation method was used to screen for decontamination efficacy. The presence of viable spores on coupons after contact with the pH-amended bleach for various contact times were determined by transferring the coupons into TSB. As described in Section 2.7, the tubes were cultured for seven days at 37 °C ± 2 °C. Results are reported as "growth" of viable spores (cloudy medium) or "no growth" (clear medium) after one day and seven days of incubation.

Counting CFU, described in Section 2.6.1, or PFU, described in Section 2.6.2, provided quantitative measurement of viable spores and viruses, respectively, after contact with pH-amended bleach for various contact times. Both the number of viable organisms recovered and the log reduction in viable organisms was reported.

Ricin was proposed for inclusion in the test matrix. However, ricin was rapidly removed from the coupons by PBS in control conditions, so the efficacy of a liquid decontaminant could not be determined. Therefore, ricin was removed from the liquid decontamination test matrices.

Table 2-7. Test Matrix for pH-Amended Bleach Investigation

Biological Agent/ Measurement	Materials and Temperatures	Test Coupon*	Positive Control Coupons†	Laboratory Blank‡	Procedural Blank'
B . anthracis **(Ames) (Qualitative Cycle Fraction)**	Glass, Painted Concrete Block, Galvanized Metal, Decorative Laminate @ 22 °C ± 2 °C	Contact times (min): 10, 20, 40, 60 min are neutralized and placed into TSB to test for viable spores Five replicate coupons per contact time	Contact times (min): 10, 20, 40, 60 min are neutralized and placed into TSB to test for viable spores Five replicate coupons per contact time	Contact times (min): 10, 20, 40, 60 min are neutralized and placed into TSB to test for viable spores One coupon per contact time	Contact times (min): 10, 20, 40, 60 min are neutralized and placed into TSB to test for viable spores One coupon per contact time
B. anthracis **(Ames) (Quantitative)**	Glass, Painted Concrete Block, Carpet, Galvanized Metal, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time
B. anthracis **(Vollum) (Quantitative)**	Glass, Painted Concrete Block, Carpet, Galvanized Metal, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time
B. anthracis **(Ames) (Quantitative)**	Glass, Painted Concrete Block, Carpet, Galvanized Metal, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 5, 10, 30 Five replicate coupons per contact time	Contact time (min): 5, 10, 30 Five replicate coupons per contact time	Contact time (min): 5, 10, 30 Five replicate coupons per contact time	Contact time (min): 5, 10, 30 Five replicate coupons per contact time
Vaccinia Virus (Quantitative)	Glass, Painted Concrete Block, Carpet, Galvanized Metal, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 0, 5 Five replicate coupons per contact time	Contact time (min): 0, 5 Five replicate coupons per contact time	Contact time (min): 0, 5 Five replicate coupons per contact time	Contact time (min): 0, 5 Five replicate coupons per contact time

*Spiked, Decontaminated; in contact with decontamination technology for specified time.
†Spiked, placed in PBS, Not Decontaminated; in contact with PBS for the specified time'
‡Not Spiked, Not Decontaminated; not in contact with liquid, but analyzed along with test and control coupons at specified time.
'Not Spiked, Decontaminated; in contact with the decontamination technology for the specified time.

2.13 Soak in Liquid ClO_2 Technology

A 1000 ppm liquid ClO_2 solution was prepared following the manufacturer's instructions by dissolving one Exterm-6 disinfectant tablet (ClorDiSys Solutions, Inc., Lebanon, NJ) in 500 mL of water. Exterm-6 disinfectant tablets contain an inorganic acid (25%-35%), sodium chlorite (15%-30%), an inorganic salt (35%-45%), and an activator (5%-10%) that generates ClO_2 when dissolved in water.

To stop the decontamination action of the liquid ClO_2 and extract the biological agent from coupons, the coupons were removed from the decontaminant and placed individually into sterile 50 mL conical vials to which 10.0 mL of sterile extraction buffer with neutralizer was added. Neutralization approaches were tested in an earlier EPA evaluation.[16] Phosphate-buffered saline with 1% sodium thiosulfate was the extraction buffer for vaccinia virus. Phosphate-buffered saline with 1% sodium thiosulfate and 0.1% Triton X-100 (Sigma) was the extraction buffer for spores. The tubes were agitated on an orbital shaker for 15 min at approximately 200 rpm at room temperature.

The liquid ClO_2 decontamination test matrix, shown in Table 2.8, used quantitative methodologies. Counting CFU, described in Section 2.6.1, or PFU, described in Section 2.6.2, provided quantitative measurement of viable spores and viruses, respectively, after contact with liquid ClO_2 for various contact times. Both the number of viable organisms recovered and the log reduction in viable organisms was reported.

Table 2-8. Test Matrix for Liquid ClO_2 Decontamination Investigation

Biological Agent/ Measurement	Materials and Temperatures	Test Coupon*	Positive Control Coupons†	Laboratory Blank‡	Procedural Blank˙
B. anthracis (Ames) (Quantitative)	Glass, Carpet, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 10, 30, 120 Five replicate coupons per contact time	Contact time (min):10, 30, 120 Five replicate coupons per contact time	Contact time (min): 10, 30, 120 Five replicate coupons per contact time	Contact time (min): 10, 30, 120 Five replicate coupons per contact time
B. anthracis (Vollum) (Quantitative)	Glass, Carpet, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time	Contact time (min): 5 Five replicate coupons per contact time
B. subtilis (Quantitative)	Glass, Carpet, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 10, 30, 120 Five replicate coupons per contact time	Contact time (min): 10, 30, 120 Five replicate coupons per contact time	Contact time (min): 10, 30, 120 Five replicate coupons per contact time	Contact time (min): 10, 30, 120 Five replicate coupons per contact time
Vaccinia Virus (Quantitative)	Glass, Carpet, Particle Board, Decorative Laminate @ 22 °C ± 2 °C	Contact time (min): 0, 5 Five replicate coupons per contact time	Contact time (min): 0, 5 Five replicate coupons per contact time	Contact time (min): 0, 5 Five replicate coupons per contact time	Contact time (min): 0, 5 Five replicate coupons per contact time

*Spiked, Decontaminated; in contact with decontamination technology for specified time.
†Spiked, placed in PBS, Not Decontaminated; in contact with PBS for the specified time.
‡Not Spiked, Not Decontaminated; not in contact with liquid, but analyzed along with test and control coupons at specified time.
˙Not Spiked, Decontaminated; in contact with the decontamination technology for the specified time.

2.14 Soak in Spor-Klenz® Hydrogen Peroxide-Peractic Acid (HP-PA) Solution

The following description of Spor-Klenz® Ready-to-Use (STERIS Corporation, Mentor, OH) is from the STERIS website (http://www.steris.com/products/view.cfm?id=253) and was not verified in this testing:

> A fast acting, liquid cold sterilant/disinfectant, filtered to 0.22 micron and specifically formulated for use in the sterilization and disinfection of hard surfaces. This product is a stabilized blend of peracetic acid, hydrogen peroxide, and acetic [acid] that provides fast, effective microbial control, including spores. It offers a low toxicity profile and requires no mixing or activation.

According to the Material Safety Data Sheet for Spor-Klenz® Ready to Use solution, the composition includes peracetic acid (0.8%), hydrogen peroxide (1.0%), and acetic acid (<10%).

The Spor-Klenz® Ready to Use solution (referenced hereinafter as Spor-Klenz® HP-PA) was used as received without dilution or other preparation against *Bacillus* spores. The decontamination process was terminated by removing coupons from the Spor-Klenz® HP-PA and placing coupons individually into sterile 50 mL conical vials containing 10.0 mL of sterile 1% catalase in Dey-Engley (neutralizing) broth. Dey-Engley broth is a standard medium used to neutralize disinfectant components in order to test for residual bacterial activity.* Neutralization approaches were tested in an earlier EPA evaluation.[16] The tubes were agitated on an orbital shaker for 15 min at approximately 200 rpm at room temperature to extract the spores.

The Spor-Klenz® HP-PA decontamination test matrix, shown in Table 2-8, used a preliminary qualitative test to identify a contact time with Spor-Klenz® HP-PA at which no viable spores were detected on any test coupons that had been inoculated with approximately 10^8 CFU of *B. anthracis* spores/coupon. The quantitative methodology, described in Section 2.6.1, measured viable, colony-forming spores recovered from coupons after contact with Spor-Klenz® HP-PA for various contact times.

Both the number of viable organisms recovered and the log reduction in viable organisms was reported as CFU.

2.15 Soak in Oxonia Active® Solution

The following description Ecolab Oxonia Active® solution is from the product label and from the Material Safety Data Sheet; this information was not verified in this testing:

> Oxonia Active® acid sanitizer is recommended for use on pre-cleaned surfaces such as

* For a further description, see: www.sigmaaldrich.com/etc/medialib/docs/Fluka/Datasheet/d3435dat.Par.0001.File.tmp/d3435dat.pdf.

equipment, tanks, vats, filters, evaporators, pasteurizers and aseptic equipment in dairies, breweries, wineries, beverage and food processing plants. This product is effective as a sanitizer when solution is prepared in water of up to 500 ppm hardness $CaCO_3$.

The label instructions specify use of a 5% solution of Oxonia Active® for sterilization. At a temperature of 20˚C, a 6-hr contact time is required. Per recommendations from the vendor, Oxonia Active® at a 7% concentration (rather than label strength) was used against *Bacillus* spores in this investigation. EPA chose to follow the vendor recommendation as to the concentration (7%) that was used in this investigation. Contact times were selected not to exceed 60 min, the maximum time considered by EPA practitioners to be practical for field application of liquid decontamination technologies.

According to the Material Safety Data Sheet for Oxonia Active®, the composition includes peracetic acid (5% - 10%), hydrogen peroxide (15% - 40%), and acetic acid (7% - 13%).

The Oxonia Active® decontamination process was terminated by removing coupons from the Oxonia Active® HP-PA solution and placing coupons individually into sterile 50 mL conical vials containing 10.0 mL of sterile 1% catalase in Dey-Engley broth. Catalase was added to the standard Dey-Engley neutralizing broth to rapidly decompose the HP. Neutralization approaches were tested in an earlier EPA evaluation.[16] The tubes were agitated on an orbital shaker for 15 min at approximately 200 rpm at room temperature to extract spores.

The Oxonia Active® test matrix is shown in Table 2-10. Test coupons were inoculated with approximately 10^8 CFU of *B. anthracis* or *B. subtilis* spores/coupon. The quantitative methodology, described in Section 2.6.1, provided quantitative measurement of viable spores after contact with Oxonia Active® HP-PA for various contact times. The decontamination process was terminated using 1% catalase in Dey-Engley broth. Both the number of viable organisms recovered and the log reduction in viable organisms was reported.

Table 2-9. Test Matrix for the Spor-Klenz® HP-PA Decontamination Investigation

Biological Agent/ Measurement	Materials and Temperatures	Test Coupon*	Positive Control Coupons†	Laboratory Blank‡	Procedural Blank˙
B. anthracis (Ames) (Qualitative)	Glass, Painted Concrete, Galvanized Metal, Decorative Laminate @ 20 °C ± 2 °C	Contact time (min): 5, 10, 20, 30 Three replicate coupons per contact time	None	None	None
B. anthracis (Ames) (Quantitative)	Glass Painted Concrete Carpet Galvanized Metal Particle Board Decorative Laminate @ 20 °C ± 2 °C	Contact time (min): 10, 20, 30 Five replicate coupons per contact time	Non-contact time (min): 10 (three replicates) 20 (one replicate) 30 (one replicate)	One coupon	Non-contact time (min): 30 (one coupon)
B. subtilis (Quantitative)	Glass @ 20 °C ± 2 °C	Contact time (min): 10, 20, 30 Five replicate coupons per contact time	Non-contact time (min): 10 (three replicates) 20 (one replicate) 30 (one replicate)	One coupon	Non-contact time (min): 30 (one coupon)

*Spiked, decontaminated.
†Spiked, placed in PBS, not decontaminated.
‡Not spiked, not decontaminated.
˙Not spiked, decontaminated.

Table 2-10. Test Matrix for the Oxonia Active® Decontamination Investigation

Biological Agent/ Measurement	Materials and Temperatures	Test Coupon*	Positive Control Coupons†	Laboratory Blank‡	Procedural Blank'
B. anthracis (Ames) (Quantitative)	Glass Painted Concrete Carpet Galvanized Metal Particle Board Decorative Laminate @ 20 °C ± 2 °C	Contact time (min): 10, 30, 60 Five replicate coupons per contact time	Non-contact time (min): 10 (three replicates) 20 (one replicate) 30 (one replicate)	One coupon	Non-contact time (min): 30 (one coupon)
B. subtilis (Quantitative)	Glass @ 20 °C ± 2 °C	Contact time (min): 10, 30, 60 Five replicate coupons per contact time	Non-contact time (min): 10 (three replicates) 20 (one replicate) 30 (one replicate)	One coupon	Non-contact time (min): 30 (one coupon)

*Spiked, decontaminated.
†Spiked, placed in PBS, not decontaminated.
‡Not spiked, not decontaminated.
'Not spiked, decontaminated.

3.0 Quality Assurance/Quality Control

QA/quality control (QC) procedures were performed in accordance with the test/QA plans and associated amendments developed for the investigations. QA/QC procedures are summarized below.

3.1 Performance Evaluation (PE) Audits

PE audits were conducted to assess the quality of the results obtained during these experiments. No PE audits were performed for biological agents and surrogates because quantitative standards for these biological materials do not exist. The confirmation procedure, controls, blanks, and method validation efforts support the biological evaluation results. For the chlorine dioxide, all of the PE audits performed were within the target tolerances specified in the test/QA plan. For liquid testing, all of the PE audits performed were within the target tolerances specified in the test/QA plan. For methyl bromide testing, all of the PE audits performed were within the target tolerances specified in the test/QA plan. The results of these analyses are given in Tables 3-1 through 3-3. All PE audit results were within the respective target tolerances.

Table 3-1. ClO_2 PE Audit Results

Parameter	Date of Audit	Value of Standard	Measured Result	Difference	Expected Tolerance
[ClO_2]	1/15/07	1000 mg/L Sodium Chlorite	1012 mg/L Sodium Chlorite	1.2%	±10%
Time	1/11/07	10:43:00‡	10:43:00‡	0 seconds	±1 min/30 days
		10:53:07‡	10:53:07‡	0 seconds	
		11:03:10‡	11:03:10‡	0 seconds	
		11:13:02‡	11:13:02‡	0 seconds	
Temperature	1/11/07	20 °C*	19.8 °C*	-0.2 °C	±1 °C
RH	1/11/07	23.5%*	17.25 %*	-6%	±10%
Microplate Reader	1/18/07	Optical density at 590 nm	Optical density at 590 nm		±1.0%
		0.269	0.270H	0.37%	
		0.539	0.539	0.00%	
		1.122	1.121	-0.09%	
		1.629	1.628	-0.06%	
		Optical density at 635 nm	Optical density at 635 nm		
		0.274	0.273	-0.36%	
		0.533	0.531	-0.38%	
		1.088	1.086	-0.18%	
		1.577	1.574	-0.19%	

*****Average of four measurements.
H All measured results for the microplate reader were an average of 24 measurements.
‡ Time in hr: min: seconds.

Table 3-2. Liquid PE Audit Results

Parameter	Date of Audit	Value of Standard	Measured Result	Difference	Expected Tolerance
Temperature – unit 1[¶]	11/16-12/13/06	22.2 °C [*]	21.7 °C [*]	0.5 °C	±1 °C
– unit 2[¶]	12/11-13/06	21.2 °C [*]	22.0 °C [*]	0.8 °C	
– unit 3[¶]	12/11-13/06	21.0 °C [*]	21.9 °C [*]	0.9 °C	
Time	11/16/06	08: 08: 05[‡]	08: 08: 05[‡]	0 seconds	±1 min/30 days
	11/21/06	09: 30: 02[‡]	09: 30: 02[‡]	0 seconds	
	12/11/06	14: 50: 36[‡]	14: 50: 36[‡]	0 seconds	
	12/11/06	14: 53: 48[‡]	14: 53: 48[‡]	0 seconds	
	12/11/06	14: 55: 02[‡]	14: 55: 02[‡]	0 seconds	
	12/13/06	15: 08: 17[‡]	15: 08: 17[‡]	0 seconds	
	12/13/06	15: 09: 32[‡]	15: 09: 32[‡]	0 seconds	
	12/13/06	15: 10: 24[‡]	15: 10: 24[‡]	0 seconds	
Microplate reader	1/18/07	Optical density at 590 nm	Optical density at 590 nm		±1.0%
		0.269	0.270[H]	0.37%	
		0.539	0.539	0.00%	
		1.122	1.121	-0.09%	
		1.629	1.628	-0.06%	
	1/18/07	Optical density at 635 nm	Optical density at 635 nm		
		0.274	0.273	-0.36%	
		0.533	0.531	-0.38%	
		1.088	1.086	-0.18%	
		1.577	1.575	-0.19%	

[*] Average of four measurements for unit 1, average of two measurements for units 2 and 3.
[H] All measured results for the microplate reader were an average of 24 measurements.
[‡] Time in hr: min: second.
[¶]Units 1-3 refers to the three sensors that were used to measure temperature during the testing.

In the audit of the manual plate count, only one repeat plate was read instead of five as required by the test/QA plan. This deviation was noted, but had no impact on the results.

3.2 Technical Systems Audit

The Battelle QA Manager conducted technical systems audits to ensure that the tests were being performed in accordance with the appropriate test/QA plans. As part of the audit, the Battelle QA Manager reviewed the reference sampling and analysis methods used, compared actual test procedures with those specified in the test/QA plan, and reviewed data acquisition and handling procedures. No significant findings were noted in these audits that might impact the quality of the evaluation results. The records concerning the technical systems audits were permanently stored with the Battelle QA Manager.

Table 3-3. MeBr PE Audit Results

Parameter	Date of Audit	Value of Standard	Measured Result	Difference	Expected Tolerance
[MeBr] *	2/15/08	49 mg/L	53 mg/L	8%	±10%
Time	5/11/07	‡	‡	0 seconds/ min	±1 min /30 days
Temperature	5/11/07	19.7 °C	21.0 °C	1.3 °C	±2 °C
RH	5/11/07	48%	48%	0.00%	±5% of full scale
Manual Plate Count[H]	8/22/07	9.47 x10^8 CFU	9.50 x 10^8 CFU	0.32%	±10% agreement in repeated plate counts

*****Average of two measurements. Prior to use, the Fumiscope was returned to the manufacturer for calibration. The Fumiscope measurements were compared to measurements with a calibrated VIG M-20 total hydrocarbon analyzer. Differences between measurements made with the total hydrocarbon analyzer and the Fumiscope meter varied up to about 20% depending on concentration of MeBr. Fumiscope readings were reported as specified in the test/QA plan to reflect readings that would be expected in field use.
[H]Only one plate was read instead of five required by test/QA plan - Noted below under deviations.
‡ 0 second/min was observed for 44 of 44 comparisons.

3.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. Battelle's QA Manager traced the data from the initial acquisition through reduction to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

QA/QC Reporting

Each assessment and audit was documented in accordance with the appropriate test/QA plan and associated amendments. For these evaluations, no significant findings were noted in any assessment or audit, and no follow-up corrective action was necessary. Copies of the TSA and assessment reports were distributed to the EPA QA Manager and Battelle staff.

3.4 Deviations from the Test QA Plans

3.4.1 General Deviation

One general deviation was documented and reported to the EPA. The test/QA plans specified that dilutions through 10^{-7} would be analyzed. In some cases, dilutions outside the useful range, based on expected concentrations of biological agent, were not run. This judgment was based on historical data and experience. For example, for positive controls only three dilutions, i.e., 10^{-3}, 10^{-4}, and 10^{-5} would be plated. For decontamination tests where no viable organisms were expected, only the undiluted solution and 10^{-1}, 10^{-2}, and 10^{-3} dilutions might be plated. In some cases with high confidence of no organisms surviving, only the undiluted extract was plated. The reduced plating saved time and money without reducing useful data. There was no impact on results.

3.4.2 Chlorine Dioxide

One deviation was documented. The test/QA plan specified that test coupons would be removed from the test chamber after given periods of exposure to the fumigant. During the cycle fractionation, 3000 ppmv ClO_2, 75% RH trials for *B. anthracis*, this removal was done by having the spiked coupons in closed vials in the test chamber and using the gloves in the glove box to open them in sequence and remove them from the vials so that an appropriate contact time for exposure to ClO_2 was achieved. Contact time was the time from opening the vial containing the spiked coupon in the fumigation test chamber until the coupon was removed from the test chamber. Time zero (T0), positive control coupons were in sealed vials in the test chamber for the full 180 min of fumigation and removed from the chamber without opening the vials; there was no exposure of the T0 coupons to the fumigant. There was no impact on the results.

3.4.3 Liquid Testing

A number of deviations were documented and described below.

One procedural blank, rather than two specified in the test/QA plan, was used at each time point. The results of the procedural blanks do not enter into the calculation of efficacy and therefore did not impact the results.

Five-min positive controls were used as T0 positive controls for spores. Spores are known to be highly persistent on the building materials used under ambient laboratory conditions so that a difference of five min in extracting the positive controls would have no impact on recoveries and, therefore, no impact on the results.

Small glass coupons were spiked with a single 10 µL drop instead of ten 1µL drops as specified in the test/QA plan. The test/QA plan was intended to require use of a single 10 µL drop instead of ten 1µL drops for the small glass coupons to be consistent with other EPA testing. The deviation is actually a correction of the test/QA plan and provides the desired results.

The cultures of *Bacillus* on TSA with no CFU observed after 18-24 hr were not returned to incubation for up to 72-hr total and re-examined per Section B.4.2.1 of the test/QA plan. The practice

of incubating for additional time has been dropped from subsequent test/QA plans. The additional incubation time was found not to be useful. There was no impact on the results.

The test/QA plan specifies that in the event of contamination of the laboratory or procedural blank, the test will be rejected. In the testing of *Bacillus subtilis* on glass decontaminated with Oxonia (6/11/09) the procedural blank extract contained a low level of contamination. In this report the data are flagged and the contamination is noted. Including the data, rather than rejecting the data, is a deviation from the test/QA plan. However, conclusions do not appear to be compromised and the data were believed to be useful and therefore were included in the report with caveats, rather than rejecting the data. The results show comparable and high decontamination efficacy, ≥5.78 log reduction, for both *B .anthracis* and *B. subtilis*. If cross contamination of the test coupons occurred in the *B. subtilis* test, *B. subtilis* decontamination may have been even higher than reported.

In the cycle fractionation, the target application range was $7.5 \times 10^7 – 1.25 \times 10^8$; the actual application used was 6.8×10^7. The qualitative cycle fractionation test was performed to guide the selection of decontamination parameters and was effective for that purpose. The results are easily and correctly interpreted based on the known number of spores applied. There was no impact on the results.

During the Spor-Klenz® and Oxonia Active® efficacy testing the target application range was $7.5 \times 10^7 – 1.25 \times 10^8$. The actual range exceeded the target range; the actual application range was $1.2 \times 10^8 – 6.9 \times 10^8$. The results are valid, but the potential dynamic range is 0.08 – 0.74 log greater with the higher applications. The comparison of the surrogate *B. subtilis* to *B. anthracis* is not impacted. Comparisons across technologies, not an objective of this report, would need to consider the dynamic range of the respective tests. There was no impact on the conclusions of this report.

During the Spor-Klenz® and Oxonia Active® efficacy testing, there were three cases in which the spore recoveries were below the acceptance range of 20% - 120%:

- Spor-Klenz® /Glass: 6% recovery
- Oxonia Active® /Laminate: 2% recovery
- Oxonia Active® /Metal: 5%.

The test/QA plan specifies that in the event of low recoveries from the positive control coupons, the test will be rejected. The tests were not rejected but the data are flagged in the results. Because the initial spore load was higher than specified in the test/QA plan (noted above), the dynamic range, even with lower than target recoveries, was sufficient to observe a 6-log reduction and observed intended results. There was minimal or no impact on the results.

3.4.4 Methyl Bromide

Two deviations were documented. The first deviation was a spore application outside of the target range. The test/QA plan stated that the number of *B. anthracis* CFU in the stock spore suspension to be spiked onto coupons will be acceptable if the application controls are within ±25% of the target

spike level. The stock suspension in Trial 8 exceeded this range. There was no impact on the results.

The second deviation covered two separate items. For the first item, the test/QA plan specified that temperature and RH in the test chamber would be monitored every 20 min during working hours using a NIST-traceable system, and monitored continuously during decontamination with the continuous monitoring system checked daily with a NIST-traceable thermometer/hygrometer. Instead, a NIST-traceable thermometer/hygrometer was used to take measurements roughly every hr during decontamination. The temperature and RH were stable so there was no impact on the results.

For the second item, five replicates were to be plated, counted, and compared with an expected agreement of ±10%. Only one replicate plate was prepared and counted during the PE audit with an agreement of ±0.32% - well below the tolerance. The personnel performing the plate counts are highly experienced and competent and are expected to have repeatable and accurate counts. There was no impact on the results.

4.0 Fumigation Results

4.1 ClO_2 Fumigation Results

Adaptive management, i.e., making changes to the test/QA plan based on knowledge gained during the investigation, resulted in trials being added to and removed from the test matrix. Results are presented in a sequence intended to provide a logical flow. However, in some cases this differs from the sequence in which the testing was performed (resulting, for example, in Trial 4g results being presented ahead of Trial 1). Trial numbers are included for ease of reference to the test matrix in Table 2-2. ClO_2 fumigation trials with the same number were performed in the same fumigation cycle.

4.1.1 Trial 4g: ClO_2 Fumigation Cycle Fractionation Results

The results of the qualitative cycle fractionation test using ClO_2 fumigation of *B. anthracis* Ames on plate glass and *B. atrophaeus* and *B. subtilis* BIs are shown in Table 4-1. At specified ClO_2 fumigation contact times, the coupons and BIs were removed from the test chamber and placed into TSB as described in Section 2.7. The BIs included: *B. atrophaeus* spores on filter paper strips in glassine (Raven Labs Division, Lakewood, CO); *B. atrophaeus* spores on filter paper strips (Raven) removed from the glassine prior to fumigation; *B. subtilis* spores on stainless steel disk in Tyvek® envelope (Apex Laboratories, Apex, NC); and *B. subtilis* spores on stainless steel disk (Apex) removed from the Tyvek® envelope prior to fumigation.

Contact for 30 min at 3000 ppmv ClO_2 killed all *B. anthracis* Ames, *B. atrophaeus*, and *B. subtilis* spores, except the *B. atrophaeus* spores on filter paper enclosed in a glassine envelope. The glassine-enclosed *B. atrophaeus* spores were killed on one of three spore strips at a 90-min contact time and were killed on all three spore strips at a 120-min contact time. Glassine paper has the characteristic of being almost impervious to water vapor (e.g., see http://www.papertecinc.com/specPaper.cfm) whereas Tyvek® is permeable to water vapor (e.g., see http://www2.dupont.com/Tyvek_Weatherization/en_US/products/residential/resi_homewrap.html). Humidity is a key parameter related to *Bacillus* species spore inactivation with ClO_2 gas (discussed later in this section).

Table 4-1. Results from Qualitative Evaluation of ClO_2 Fumigation of *B. anthracis* Ames Spores and Surrogates

Bacterium and Material (Trial 4g)	[ClO_2], ppmv	Temperature, RH	Positive Control Coupon	Decontaminated, Contact Time 30 min	60 min	90 min	120 min
B. anthracis Ames, Plate Glass	3000	25 - 26 °C 71 - 74%	+	-	-	-	-
B. atrophaeus spores on filter paper strips in glassine (Raven)	3000	25 - 26 °C 71 - 74%	+	+	+	+/- (2 +, 1 -)	-
B. atrophaeus spore on filter paper strips not in glassine (Raven)	3000	25 - 26 °C 71 - 74%	+	-	-	-	-
B. subtilis spores on stainless disk in Tyvek® envelope (Apex)	3000	25 - 26 °C 71 - 74%	+	-	-	-	-
B. subtilis spores on stainless disk not in Tyvek® envelope (Apex)	3000	25 - 26 °C 71 - 74%	+	-	-	-	-

"+" indicates that all replicates (n = 3) are positive for growth after incubation at 37 °C for seven days; *B. anthracis* confirmed morphologically by plating onto tryptic soy monoplates.
"-" indicates that all replicates (n = 3) are negative for growth after incubation at 37 °C for seven days; absence of *B. anthracis* confirmed morphologically by plating onto tryptic soy monoplates.
"+/-" indicates that two replicates were positive and one replicate was negative for growth after incubation at 37 °C for seven days; *B. anthracis* confirmed morphologically by plating onto tryptic soy monoplates.
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

4.1.2 Trials 4a and 4b: Fumigation Cycle Fractionation Results for Two Concentrations of ClO_2 against B. anthracis

The results of the qualitative cycle fractionation test using ClO_2 fumigation of *B. anthracis* Ames spores are shown in Table 4-2. The qualitative cycle fractionation test was used to indicate the contact time required at two target concentrations of ClO_2 (750 ppmv and 3000 ppmv) for complete kill (no observed CFU) of *B. anthracis* Ames spores on coupons of various building materials. Three test coupons of each material type, spiked with *B. anthracis* spores, were exposed to ClO_2 for each specified contact time.

The T0 positive control and test coupons were placed in glass tubes, sealed, and placed into the decontamination chamber during the fumigation cycle. Test coupons were opened and the coupons removed from the glass tube and exposed to fumigant in the test chamber at appropriate intervals, e.g., the tube for a 40-min contact time exposure would be opened and the coupon exposed to the fumigant 40 min prior to aeration.

After the fumigation cycle was completed, the T0 coupons, still in sealed glass tubes, were removed from the test chamber, removed from the glass tube, and cultured in TSB to detect viable spores. All T0 coupons, except particle board, exhibited growth in TSB. The particle board coupons did not exhibit growth in TSB. However, subculture of an aliquot of the TSB containing the particle board coupons onto tryptic soy agar plates resulted in growth of *B. anthracis* Ames. (Colony morphology was consistent with *B.*

anthracis Ames and no colonies were recovered from procedural blank coupons.) The particle board appears to have biostatic properties that inhibited the growth of *B. anthracis* Ames in the TSB. As a result of this interference, no conclusions can be reached on the effectiveness of ClO_2 fumigation against *B. anthracis* Ames on particle board using the qualitative cycle fractionation test. Therefore the particle board was not included in the testing at 3000 ppmv.

Table 4-2. Results from Qualitative Evaluation of ClO_2 Fumigation of *B. anthracis* Ames Spores

Material	$[ClO_2]$, ppmv	Mean Temperature, Mean RH	Decontaminated, Contact Time						
***B. anthracis* Ames**			**0 min***	**20 min**	**40 min**	**60 min**	**80 min**	**100 min**	**120 min**
Glass	750	24.7 °C 82.9%	+	+	+	+	-	-	-
Painted Concrete	750	24.7 °C 82.9%	+	+	+	-	-	-	-
Galvanized Metal	750	24.7 °C 82.9%	+	+	+	+	+	+	+
Decorative Laminate	750	24.7 °C 82.9%	+	+	+	+	-	-	-
Cellulose Insulation	750	24.7 °C 82.9%	+	+	+	+	+	+	+
Particle Board	750	24.7 °C 82.9%	- (+)	-	-	-	-	-	-
***B. anthracis* Ames**			**0 min***	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
Glass	3000	24.6 °C 78.9%	+	-	-	-	-	-	-
Painted Concrete	3000	24.6 °C 78.9%	+	-	-	-	-	-	-
Galvanized Metal	3000	24.6 °C 78.9%	+	-	-	-	-	-	-
Decorative Laminate	3000	24.6 °C 78.9%	+	-	-	-	-	-	-
Cellulose Insulation	3000	24.6 °C 78.9%	+	+	+	+	+	+	+

"+" indicates that all replicates (n = 3) are positive for growth after incubation at 37 °C for seven days; *B. anthracis* confirmed morphologically by plating onto tryptic soy monoplates.
"-" indicates that all replicates (n = 3) are negative for growth after incubation at 37 °C for seven days; absence of *B. anthracis* confirmed morphologically by plating onto tryptic soy monoplates.
"- (+)" indicates that all replicates (n = 3) are negative for growth after incubation at 37 °C for seven days, however, *B. anthracis* colonies grew, and were confirmed morphologically, when plated onto tryptic soy monoplates.
* These inoculated coupons were in sealed tubes in the decontamination chamber during fumigation.
Note $[ClO_2]$ is concentration of ClO_2 in the chamber atmosphere, in ppmv.

The results of the qualitative testing showed that the ClO_2 concentration and contact time necessary for complete kill of *B. anthracis* Ames spores (negative for growth) depends on the type of material to which the spores were applied. At 750 ppmv ClO_2, complete kill of *B. anthracis* Ames spores (negative for growth) was observed in as little as 60 min for painted concrete coupons, and 80 min for glass and decorative laminate. In contrast, viable *B. anthracis* Ames spores were still observed on galvanized metal and compressed cellulose insulation after a 120-min contact with 750 ppmv ClO_2.

At 3000 ppmv ClO_2, complete kill of *B. anthracis* Ames spores (negative for growth) was observed in 10 min for all coupon types except compressed cellulose insulation. Viable *B. anthracis* Ames spores were still observed on cellulose insulation after 60-min contact with 3000 ppmv ClO_2.

4.1.3 B. anthracis on Painted Concrete: Quantitative CT Investigation

Trials 1-3 investigated the efficacy of ClO_2 against *B. anthracis* Ames spores on painted concrete using the quantitative method (Section 2.6) at three CT values at high RH (>80%). The three CT values, 2250 ppmv-hr, 4500 ppmv-hr, and 9000 ppmv-hr, were repeated at three different ClO_2 concentrations: 3000 ppmv, 1500 ppmv and 750 ppmv. The quantitative recovery results for *B. anthracis* Ames spores on painted concrete exposed to ClO_2 at specified concentrations and contact times are shown in Tables 4-3 and 4-4. Complete kill (>7 log reduction) was observed at all CT values (2250 ppmv-hr and higher) and at all ClO_2 concentrations tested (750 ppmv and higher).

Table 4-3. *B. anthracis* Ames Spores CFU after ClO_2 Fumigation at Various CTs

Trial	Biological Agent, Material	[ClO_2], Temperature, RH	T0† Mean CFU (SD)	Positive Controls			Decontaminated Coupons*		
				Time, Mean CFU (SD)	Time, Mean CFU (SD)	Time, Mean CFU (SD)	CT, Contact time, Mean CFU	CT, Contact time, Mean CFU	CT, Contact time, Mean CFU
1	*B. anthracis* Ames, Painted Concrete	3000 ppmv 24 - 25 °C 89 - 91%	6.57×10^7 (7.39×10^6)	0.75 hr, 5.99×10^7 (1.15×10^7)	1.5 hr, 5.67×10^7 (1.36×10^7)	3 hr, 7.18×10^7 (1.64×10^7)	2250 ppmv-hr 0.75 hr, 0	4500 ppmv-hr, 1.5 hr, 0	4500 ppmv-hr, 3 hr, 0
2	*B. anthracis* Ames, Painted Concrete	1500 ppmv 24 - 25 °C 81 - 87%	6.21×10^7 (2.21×10^7)	1.5 hr, 7.86×10^7 (6.25×10^6)	3 hr, 6.96×10^7 (1.09×10^7)	6 hr, 5.58×10^7 (1.02×10^7)	2250 ppmv-hr 1.5 hr, 0	4500 ppmv-hr, 3 hr, 0	4500 ppmv-hr, 6 hr, 0
3	*B. anthracis* Ames, Painted Concrete	750 ppmv 24 - 25 °C 72 - 82%	7.50×10^7 (1.38×10^7)	3 hr, 6.93×10^7 (6.06×10^6)	6 hr, 7.21×10^7 (1.25×10^7)	12 hr, 6.34×10^7 (9.98×10^6)	2250 ppmv-hr 3 hr, 0	4500 ppmv-hr, 6 hr, 0	4500 ppmv-hr, 12 hr, 0

*SD not calculated when no spores are recovered from any test coupon
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 4-4. Mean Log Reduction of *B. anthracis* Ames Spores after ClO_2 Fumigation of Painted Concrete at Various CTs

Trial	CT, ppmv-hr	[ClO_2], ppmv	Temperature, °C	RH, %	Time, hr	Mean Log Reduction and p-value[*, †]
1	2,250	3000	24 - 25	89 - 91	0.75	**>7.77 (5/5) p=0.0135**
	4,500				1.5	**>7.75 (5/5) p=0.0135**
	9,000				3	**>7.85 (5/5) p=0.0135**
2	2,250	1500	24 - 25	81 - 87	1.5	**>7.89 (5/5) p=0.0135**
	4,500				3	**>7.84 (5/5) p=0.0135**
	9,000				6	**>7.74 (5/5) p=0.0135**
3	2,250	750	24 - 25	72 - 82	3	**>7.84 (5/5) p=0.0135**
	4,500				6	**>7.85 (5/5) p=0.0135**
	9,000				12	**>7.80 (5/5) p=0.0135**

[*]Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.
[†]One or more of treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one CFU substituted for all zero recovery coupon values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicates statistically significantly greater reduction in the treated group than in the controls.
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

4.1.4 B. anthracis on Eight Materials: Quantitative CT Investigation

In Trials 4, 5, and 6, the efficacy of ClO_2 fumigation was evaluated against *B. anthracis* Ames spores on coupons of eight materials using the quantitative method (Section 2.6). All test and positive control coupons (except the small glass coupons) were inoculated with 1x 10^8 spores; the small glass coupons were inoculated with a single droplet of 10^7 spores. The results, shown in Tables 4-5 and 4-6, include ClO_2 fumigations at two CT values and at two temperatures.

At 3000 ppmv for 3 hr (9000 ppmv-hr) (24 - 25 °C and 85 - 95% RH), no viable spores were observed on any material coupons except for one of the five painted concrete coupons and all of the coupons of compressed cellulose insulation. The results correspond to a >6 log reduction in viable spores for all fumigated materials except cellulose insulation.

Repeating the 9000 ppmv-hr test at a higher temperature and essentially the same RH (30 - 32 °C and 74 - 89% RH) resulted in no CFU being observed on any fumigated material, including painted concrete and cellulose insulation. These results correspond to a >6 log reduction in viable spores for all fumigated materials.

Table 4-5. *B. anthracis* Ames Spores Mean CFU after ClO_2 Fumigation at Various CTs, Temperatures

Trial	*B. anthracis* Ames	[ClO_2], Contact Time, CT	Temperature Range	RH Range	Control Mean CFU (SD)	Decon Mean CFU (SD) *
4	Glass (small), 1x 10^7 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	4.70 x 10^6 (1.13 x 10^6)	0
4	Painted Concrete, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	3.89 x 10^7 (1.18 x 10^7)	6.61 x 10^2 (1.48 x 10^3) [1 +, 4 -]
4	Galvanized Metal, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	4.68 x 10^6 (2.00 x 10^6)	0
4	Decorative Laminate, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	3.58 x 10^7 (1.18 x 10^7)	0
4	Cellulose Insulation, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	4.83 x 10^7 (2.13 x 10^7)	5.07 x 10^2 (3.24 x 10^2) [5 +]
4	Particle Board, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	3.53 x 10^6 (3.25 x 10^6)†	0
4	Industrial Carpet, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	4.31 x 10^7 (8.43 x 10^6)	0
4	Plate Glass, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	24 - 25 °C	85 - 95%	3.90 x 10^7 (9.24 x 10^6)	0
5	Glass (small), 1x 10^7 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	3.42 x 10^6 (6.98 x 10^5)	0
5	Painted Concrete, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	5.29 x 10^7 (2.33 x 10^7)	0
5	Galvanized Metal, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	9.19 x 10^6 (1.35 x 10^7)	0
5	Decorative Laminate, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	1.07 x 10^8 (9.03 x 10^7)	0
5	Cellulose Insulation, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	1.17 x 10^8 (1.59 x 10^8)	0
5	Particle Board, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	4.02 x 10^7 (1.39 x 10^7)	0
5	Industrial Carpet, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	5.42 x 10^7 (2.64 x 10^7)	0

*SD not calculated when no spores are recovered from any test coupon.
†Two of the five control coupons had no recovered agent.

Table 4-5. Continued

Trial	*B. anthracis* Ames	[ClO_2], Contact Time, CT	Temperature Range	RH Range	Control Mean CFU (SD)	Test Mean CFU (SD) *
5	Plate Glass, 1x 10^8 spores applied	3000 ppmv, 3 hr, 9000 ppmv-hr	30 - 32 °C	74 - 89%	6.50 x 10^7 (3.82 x 10^7)	0
6	Glass (small), 1x 10^7 spores applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	4.01 x 10^6 (1.63 x 10^6)	0
6	Painted Concrete, 1x 10^8 spores applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	8.13 x 10^7 (2.82 x 10^7)	0
6	Galvanized Metal, 1x 10^8 spores Applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	2.35 x 10^7 (1.92 x 10^7)	0
6	Decorative Laminate, 1x 10^8 spores Applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	7.63 x 10^7 (2.23 x 10^7)	0
6	Cellulose Insulation, 1x 10^8 spores Applied	3000 ppmv, 4 hr, 12,000 ppmv-hr	25 - 27 °C	83 - 97%	9.07 x 10^7 (1.18x 10^7)	0
6	Particle Board, 1x 10^8 spores Applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	4.55 x 10^7 (2.27 x 10^7)	0
6	Industrial Carpet, 1x 10^8 spores Applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	6.27 x 10^7 (3.64 x 10^6)	0
6	Plate Glass, 1x 10^8 spores Applied	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	7.32 x 10^7 (8.02 x 10^6)	0

*SD not calculated when no spores are recovered from any test coupon.
†Two of the five control coupons had no recovered agent.
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 4-6. Mean Log Reduction of *B. anthracis* Ames Spores after ClO_2 Fumigation at Various Temperatures and Contact Times

Trial	CT, ppmv-hr	[ClO_2] ppmv	Time, hr	Material	Mean Log Reduction (95% Confidence Interval) and p-value[*H]
4	9000	3000	3	Glass (small)	**>6.66 (5/5) p=0.0135**
				Painted Concrete	**>6.87 (4/5) p=0.0135**
				Galvanized Metal	**>6.64 (5/5) p=0.0135**
				Decorative Laminate	**>7.53 (5/5) p=0.0135**
				Cellulose Insulation	**5.03 (4.67, 5.39) p<0.0001**
				Particle Board	Control Data Failed Acceptance Criteria[t]
				Industrial Carpet	**>7.63 (5/5) p=0.0135**
				Plate Glass	**>7.58 (5/5) p=0.0135**
5	9000	3000	3	Glass (small)	**>6.53 (5/5) p=0.0135**
				Painted Concrete	**>7.69 (5/5) p=0.0135**
				Galvanized Metal	**>6.54 (5/5) p=0.0135**
				Decorative Laminate	**>7.92 (5/5) p=0.0135**
				Cellulose Insulation	**>7.73 (5/5) p=0.0135**
				Particle Board	**>7.58 (5/5) p=0.0135**
				Industrial Carpet	**>7.69 (5/5) p=0.0135**
				Plate Glass	**>7.75 (5/5) p=0.0135**
6	1000	3000	0.33	Glass (small)	**>6.58 (5/5) p=0.0135**
				Painted Concrete	**>7.89 (5/5) p=0.0135**
				Galvanized Metal	**>7.26 (5/5) p=0.0135**
				Decorative Laminate	**>7.87 (5/5) p=0.0135**
				Particle Board	**>7.62 (5/5) p=0.0135**
				Industrial Carpet	**>7.80 (5/5) p=0.0135**
				Plate Glass	**>7.86 (5/5) p=0.0135**
	12,000	3000	4	Cellulose Insulation	**>7.95 (5/5) p=0.0135**

*Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.

[†]One or more of treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one CFU substituted for all zero recovery coupon values to permit calculation of the log. For these trials, the test of statistical significance is a non-parametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicate statistically significantly greater reduction in the treated group than in the controls.

[t]Two of the five control coupons as well as all five of the treated coupons had no recovered agent.

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

The efficacy of ClO_2 fumigation against *B. anthracis* Ames spores on seven types of material coupons (all types except cellulose insulation) was evaluated at a lower CT of 1000 ppmv-hr (3000 ppmv for 0.33 hr) (25 - 27 °C and 83 - 97% RH). At the CT of 1000 ppmv-hr, no CFU were observed on any fumigated material. These results correspond to a >6 log reduction in viable spores for all fumigated materials.

Because ClO_2 did not kill all spores at a CT of 9000 ppmv-hr (at 24 - 25 °C), a

higher CT of ClO_2 fumigation was applied against *B. anthracis* Ames spores on cellulose insulation as an extra test in Trial 6. At the higher CT, 12,000 ppmv-hr (3000 ppmv for 4 hr), at 25 - 27 °C and 83 - 97% RH, no CFU were observed on fumigated cellulose insulation coupons. These results correspond to a >7 log reduction in viable spores for fumigated cellulose insulation coupons. Note that increased efficacy was observed both at a higher temperature (30 - 32 °C), a higher RH range, and a higher CT.

Test coupons were in sealed vials in the test chamber. The test coupons were removed from the vials and exposed to the fumigant for the specified contact time. For example, a 1-hr contact time would begin by removing the test coupon from the sealed vial one hr before the termination of the fumigation. Controls were used to check whether ClO_2 could leak into the sealed vials in the test chamber containing the test coupons before the contact period began (test coupons removed from the sealed vials). These controls included both positive control coupons and a potassium iodide buffer ("impinger solution") chemical indicator solution. Time zero positive control coupons were in sealed vials in the test chamber for the full fumigation cycle and removed from the chamber without opening the vials; there was no exposure of the T0 coupons to the fumigant. The positive control coupons were extracted and cultured to determine the CFU indicative of viable spores. No loss of viable spores (CFU) from T0 coupons was observed, confirming that spores in the sealed vials were not exposed to ClO_2 fumigation prior to the vials being opened.

Also, a sealed vial of potassium iodide buffer was placed into the test chamber for a full fumigation cycle at 3000 ppmv ClO_2. After the exposure, the potassium iodide solution was then shaken and checked for any color change that would be expected if ClO_2 leaked into the vial. No detectable ClO_2 was found in the potassium iodide buffer, again confirming that there was no leakage of ClO_2 into the sealed vials. The sealed vials were identical to those used to protect test coupons from ClO_2 before the vials were opened and the test coupons removed to begin the contact time.

4.1.5 Investigation of Effect of Temperature on ClO_2 Efficacy

Efficacy of fumigation with ClO_2 was compared at two temperatures. Except for cellulose insulation, no viable spores (zero CFU) were recovered from a replicate set of test coupons after fumigation at a CT of 9000 ppmv-hr at either 24 °C (Trial 4) or 30 °C (Trial 5). While it was possible to compare the control and decontamination results within such a test (with no recovered CFU) to determine a conservative estimate of effectiveness, the comparison of two such decontamination treatments to each other will yield an indeterminate result.

Only for cellulose insulation could a comparison be made. Cellulose insulation decontaminated with 3000 ppmv for 3 hr at 24 °C in Trial 4 exhibited a mean reduction of 5.03 log of *B. anthracis* Ames. In Trial 5, with 3000 ppmv for 3 hr at 32 °C, all the decontaminated cellulose insulation coupons exhibited zero remaining CFU, resulting in a minimum 7.73 mean log

reduction. The higher temperature trial was thus more efficacious by a minimum 2.70 log than the lower temperature trial and this difference was statistically significant (p=0.0135), as shown in Table 4-7. The results of this comparison must be interpreted with caution, though, since the difference between two test conditions may be due to differences in the RH range or trial-to-trial test differences as well as true differences in decontamination effectiveness.

Table 4-7. Statistical Comparison of ClO_2 Efficacy against *B. anthracis* Ames Spores on Cellulose Insulation at Two Temperatures

Comparison	Difference in Mean Log Reduction in 30°C and 24°C and p-value*
Trial 5 Cellulose at 30 °C vs. Trial 4 Cellulose at 24 °C	**>2.70 p=0.0135**

*Difference in mean efficacy at 30 °C and 24 °C. Confidence intervals and p-values are from two sample t-tests comparing mean efficacy at 30 °C and 24 °C. Bolded results are statistically significant at 0.05 level.

4.1.6 Investigation of Effect of Organic Burden, Spore Types, and RH on ClO_2 Efficacy

In cooperation with EPA, the U.S. Army Research ECBC has performed parallel fumigation studies, but with differences: EPA/ECBC used a different strain of *B. anthracis*, specifically NNR1Δ1, used different methods of spore preparation, different types of material coupons, different sizes of coupons, different RH control range, and added organic burden. In the series of trials shown in Tables 4-8 and 4-9, the following questions were addressed:

- Does the choice of strain of *B. anthracis* (Ames or NNR1Δ1) impact efficacy results?
- Does lowering the RH from a range of 80 - 84% to 75 - 77% impact efficacy of ClO_2 fumigation?
- Does the addition of organic burden to the *B. anthracis* Ames spore preparation in the EPA/ECBC methods change the decontamination efficacy of ClO_2 fumigation?

To evaluate the impact of low levels of added organic burden, the efficacy of ClO_2 fumigation was evaluated against *B. anthracis* Ames spores on I-beam steel, bare pine wood, and ceiling tile coupons using the quantitative method. Conditions were a CT of 3000 ppmv-hr (3000 ppmv for 1 hr), 27 °C, and 82 - 84% RH. In Trial 4c, no additional organic burden was added to the stock spore suspension. In Trial 4d, 0.5% fetal bovine serum was added to the stock spore suspension. No significant differences between spores with and without added organic burden were observed. Both with and without added organic burden, about 400 spores were recovered from coupons of ceiling tile and no viable spores were recovered after treatment from coupons of I-beam steel and ceiling tile.

To evaluate the impact of slightly (about 5%) lower RH, the efficacy of ClO_2 fumigation was evaluated against *B. anthracis* Ames spores on ceiling tile coupons using the quantitative method**.** Conditions in Trial 4e were 3000 ppmv-hr CT (3000 ppmv for 1 hr), 24 - 26 °C and 71 - 77% RH. The Trial 4e results at

lower RH could be compared to the results from Trial 4d at higher RH and with added organic burden. No moisture was present in the test chamber at either the higher or lower RH conditions. At the lower RH the gloves did not feel "slick" as they do when wet.

Table 4-8. Mean ClO_2 Fumigation of *B. anthracis* Ames and NNR1Δ1 Spores CFU with and without Added Organic Burden

***B. anthracis* Ames (Trial 4c)**	**[ClO_2], Contact Time, CT**	**Temperature**	**RH**	**Control Mean CFU (SD)**	**Test Mean CFU (SD)†**
I-Beam Steel	3000 ppmv, 1 hr, 3000 ppmv-hr	26 °C	80%	3.87×10^7 (1.18×10^7)	0
Bare Pine Wood	3000 ppmv, 1 hr, 3000 ppmv-hr	26 °C	80%	1.27×10^7 (8.04×10^6)	4.35×10^2 (9.70×10^2) [1 +]
Ceiling Tile	3000 ppmv, 1 hr, 3000 ppmv-hr	26 °C	80%	1.98×10^7 (7.29×10^6)	0
***B. anthracis* Ames + 0.5% Fetal Bovine Serum (Trial 4d)**	**[ClO_2], Contact Time, CT**	**Temperature**	**RH**	**Control Mean (SD)**	**Test Mean (SD)**
I-Beam Steel	3000 ppmv, 1 hr, 3000 ppmv-hr	27 °C	82 - 84%	$6.56 \times 10^{6*}$ (1.46×10^7)	0
Bare Pine Wood	3000 ppmv, 1 hr, 3000 ppmv-hr	27 °C	82 - 84%	1.41×10^7 (7.18×10^6)	4.35×10^2 (9.70×10^2) [1 +]
Ceiling Tile	3000 ppmv, 1 hr, 3000 ppmv-hr	27 °C	82 - 84%	1.58×10^7 (6.55×10^6)	0
***B. anthracis* + 0.5% Fetal Bovine Serum (Trial 4e)**	**[ClO_2], Contact Time, CT**	**Temperature**	**RH**	**Control Mean (SD)**	**Test Mean (SD)**
I-Beam Steel (NNR1Δ1 strain)	3000 ppmv, 1 hr, 3000 ppmv-hr	24 - 26 °C	71 - 77%	7.97×10^5 (2.24×10^5)	4.74×10^1 (1.04×10^2) [1 +]
Bare Pine Wood (NNR1Δ1 strain)	3000 ppmv, 1 hr, 3000 ppmv-hr	24 - 26 °C	71 - 77%	1.27×10^6 (8.90×10^5)	9.00×10^3 (1.04×10^2)
Ceiling Tile (NNR1Δ1 strain)	3000 ppmv, 1 hr, 3000 ppmv-hr	24 - 26 °C	71 - 77%	1.20×10^6 (4.47×10^5)	2.02×10^3 (1.36×10^3)
Ceiling Tile (Ames strain)	3000 ppmv, 1 hr, 3000 ppmv-hr	24 - 26 °C	71 - 77%	7.70×10^6 (3.28×10^6)	3.36×10^4 (3.36×10^4) [3 +]

*Two of the five control coupons as well as all five of the treated coupons had no recovered agent.
†SD not calculated when no spores are recovered from any test coupon.
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 4-9. Mean Log Reduction of ClO_2 Fumigation of *B. anthracis* Ames and NNR1Δ1 Spores with and without Added Organic Burden

Trial	Biological Agent	[ClO_2], Temperature RH	Time, hr	Material	Mean Log Reduction (95% Confidence Interval) and p-value[*H]
4c	Ames strain	3000 ppmv 25 °C 80%	1	I-Beam Steel	**>7.57 (5/5) p=0.0135**
				Bare Pine Wood	**>6.38 (4/5) p=0.0135**
				Ceiling Tile	**>7.27 (5/5) p=0.0135**
4d	Ames + organic burden	3000 ppmv 27 °C 82 - 84%	1	I-Beam Steel	Control Data Failed Acceptance Criteria[t]
				Bare Pine Wood	**>6.44 (4/5) p=0.0135**
				Ceiling Tile	**>7.17 (5/5) p=0.0135**
4e	Ames strain	3000 ppmv 24 - 26 °C 71 - 77%	1	Ceiling Tile	**>4.02 (2/5) p=0.0135**
	NNR1Δ1 strain	3000 ppmv 24 - 26 °C 75 - 77%	1	I-Beam Steel	**>5.41 (4/5) p=0.0135**
				Bare Pine Wood	**2.38 (1.65, 3.11) p=0.0004**
				Ceiling Tile	**2.82 (2.44, 3.21) p<0.0001**

[*]Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.

[H] One or more treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one CFU substituted for all zero recovery coupon values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicate statistically significantly greater reduction in the treated group than in the controls.

[t]Two of the five control coupons as well as all five of the treated coupons had no recovered agent.

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Trials 4c and 4d showed that low levels of organic burden added to spores had no impact on ClO_2 fumigation efficacy under the conditions tested. There was no statistically significant observed effect of adding 0.5% fetal bovine serum to the stock solution of *B. anthracis* Ames spores before applying spores to bare pine wood or ceiling tile coupons prior to fumigation with ClO_2 at 3000 ppmv-hr. However, with complete decontamination (zero CFU recovered after fumigation) it cannot be assumed that with alternate treatment conditions (lower CT) the organic burden would not impact the efficacy results. Because of failure of control coupons to meet acceptance criteria (two control coupons showed no growth), the decontamination efficacy of ClO_2 against spores on I-beam steel was not determined.

In cases where treatments being compared both result in complete decontamination, comparisons of efficacy are indeterminate. Most of the conditions for Trials 4c and 4d resulted in complete decontamination (zero CFU recovered after fumigation) and comparisons are therefore indeterminate. The exceptions are the two Trial 4e results with decontamination of

NNR1Δ1 on ceiling tile and on bare pine wood shown in Table 4-10. Within Trial 4e, the mean decontamination efficacy against *B. anthracis* Ames for ceiling tile was not significantly different from NNR1Δ1; data variability associated with the mean log reduction of *B. anthracis* Ames for ceiling tile potentially inhibited a statistically significant difference from being detected.

(The differences in efficacy attributed to the *B. anthracis* spore strain [Ames versus NNR1Δ1] might, alternatively, be attributable to differences in spore preparation methods used for the two types of spores. The potential impact of differences in the methods used to prepare spores is an alternative source of variability in spore resilience that merits further investigation. In either case, the efficacy observed for the *B. anthracis* Ames spore preparation was not significantly different from efficacy observed for the *B. anthracis* NNR1Δ1 spore preparation.)

However, comparing the log reduction in *B. anthracis* Ames on ceiling tile in Trial 4d (higher RH) with *B. anthracis* Ames on ceiling tile in Trial 4e (lower RH) showed that efficacy was about 3.3 log less at the lower RH. Thus, RH rather than the strain of *B. anthracis* appears to account for the difference in efficacy between the EPA/ECBC results and the Battelle results. The tighter RH range has a significant impact on efficacy and is a parameter that must be controlled and adequately monitored to appropriately explain efficacy testing results.

Shown in Table 4-9, efficacy was reduced at the lower RH. Viable spores were recovered from three of the five ceiling tile coupons at the lower RH (average recovery about 3000 spores). In contrast, no viable spores were recovered from any ceiling tile coupons at the higher RH in Trial 4d. At the high RH, a 7.2 log reduction of viable *B. anthracis* Ames spores from ceiling tile was observed. However, a 4.0 log reduction was observed at the low RH.

Further, after a 3000 ppmv-hr fumigation at the lower RH in Trial 4e, viable *B. anthracis* NNR1Δ1 spores were recovered from one of five coupons of I-beam steel and, on average, thousands of viable spores were recovered from all bare pine wood and ceiling tile coupons. The efficacy of ClO_2 at the lower RH (71 - 77%) ranged from a 2.4 log kill on bare pine wood to >5.4 log kill on steel I-beam.

Shown in Table 4-10, compared to *B. anthracis* Ames in Trial 4d (high RH), the mean decontamination efficacy for the *B. anthracis* NNR1Δ1 strain on bare pine wood and ceiling tile was estimated to be at least 4.1 and 4.4 log less, respectively, at the lower RH in Trial 4e. In both cases, the difference was statistically significant.

These trials comparing the EPA/Battelle testing and the EPA/ECBC testing were intended only to identify the cause(s) of differences observed in the two test approaches at one CT treatment. Based on this limited investigation, differences in results between EPA/ECBC testing and the EPA/Battelle testing at the specific CT were not shown to be due to the difference in organic burden nor the type of spore/spore preparation. However a significant difference was found for differences in RH. The higher

RH, 80% and higher, resulted in much higher efficacy (mean log reduction). A potential explanation is that at RH of 80% and higher, condensation tends to be observed. Absorption of ClO_2 into the condensate may convert the fumigation into a liquid decontamination with different properties. The results showing the extreme impact of RH on the efficacy of ClO_2 are supported by additional studies conducted by EPA/ECBC.[17] These results should not be generalized to infer that the addition of organic burden, alternative spore strains, or alternative spore preparations has no effect on efficacy results.

Table 4-10. Statistical Comparison of ClO_2 Fumigation of *B. anthracis* Ames and NNR1Δ1 Spores with and without Added Organic Burden

Comparison	Difference in Mean Log Reduction and p-value*[H]
Trial 4e Ceiling Tile (*B. anthracis* Ames) vs. Trial 4e Ceiling Tile (*B. anthracis* NNR1Δ1)	Not significant
Trial 4d Bare Pine Wood (*B. anthracis* Ames + 0.5% FBS Organic burden) vs. Trial 4e Bare Pine Wood (*B. anthracis* NNR1Δ1)	**>4.06 p=0.0135**
Trial 4d Ceiling Tile (*B. anthracis* Ames + 0.5% FBS Organic burden, high RH) vs. Trial 4e Ceiling Tile (*B. anthracis* NNR1Δ1 + 0.5% FBS Organic burden, lower RH)	**>4.35 p=0.0135**

*Difference in mean efficacy of paired test conditions. Confidence intervals and p-values are from two sample t-tests comparing mean efficacy results from two test conditions. Bolded results are statistically significant at 0.05 level.

[H] One or more treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one CFU substituted for all zero recovery coupon values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicates statistically significantly greater reduction in the treated group than in the controls.

4.1.7 ClO_2 Fumigation of Ricin at Two CTs

Trials 9 and 10 evaluated the efficacy of ClO_2 fumigation at two CT values against ricin toxin (~25 µg per test coupon) applied to seven types of material coupons. The two CT values tested were 500 ppmv-hr (1500 ppmv for 20 min) and 100 ppmv-hr (200 ppmv for 30 min). During fumigation the temperature was maintained at 23 - 25 °C and the RH was controlled at 80 - 84%. The test parameters and results are shown in Tables 4-11 and 4-12. A ClO_2 fumigation level of 500 ppmv-hr resulted in >99.1% geometric mean reduction in ricin recovered from all materials except cellulose insulation within 20 min; a 92.7% reduction in ricin from cellulose insulation was observed.

A ClO_2 fumigation level of 100 ppmv-hr resulted in >99% geometric mean reduction in ricin recovered from glass, painted concrete, and decorative laminate; lower levels of decontamination were observed for galvanized metal (98.5% reduction), cellulose insulation (93.4% reduction), particle board (95.8% reduction), and industrial carpet (98.3% reduction).

Comparing the ricin removal for the 100 and 500 ppmv-hr test conditions showed that the results were not significant for three materials. Smal but statistically significant differences were observed for the other four materials. In two cases the recoveries at the higher CT showed that the decontamination was more efficacious; in the other two cases decontamination at the lower CT were slightly more efficacious. These small differences are likely due to trial to trial differences such as differences in the recovered mass of ricin from the positive control coupons in the two trials, rather than a difference that could be attributed to differences in operational conditions.

Table 4-11. Ricin Cytotoxicity with and without ClO_2 Fumigation at Various CTs

Trial	Material	Contact Time, min	[ClO_2], ppmv	Temperature, °C	RH, %	T0 Control Mean (SD), µg	20 or 30 min Control Mean (SD), µg	Test Mean (SD), µg
9	Glass (small)	20	1500	23 - 25	80 - 84	2.56 (1.17)	4.88 (1.62)	0.01 (0.002)
9	Painted Concrete	20	1500	23 - 25	80 - 84	17.58 (4.02)	27.10 (5.13)	0.01 (0.002)
9	Galvanized Metal	20	1500	23 - 25	80 - 84	2.97 (0.56)	1.92 (1.20)	0.01 (0.009)
9	Decorative Laminate	20	1500	23 - 25	80 - 84%	0.70 (0.69)	1.21 (1.25)	0.01 (0.001)
9	Cellulose Insulation	20	1500	23 - 25	80 - 84%	2.33 (0.87)	3.22 (1.54)	0.20 (0.07)
9	Particle Board	20	1500	23 - 25	80 - 84%	1.24 (0.67)	4.75 (0.80)	0.03 (0.005)
9	Industrial Carpet	20	1500	23 - 25	80 - 84%	19.36 (3.34)	21.46 (4.80)	0.09 (0.03)
10	Glass (small)	30	200	23 - 25	80 - 84%	4.25 (2.33)	4.92 (0.97)	0.01 (0.002)
10	Painted Concrete	30	200	23 - 25	80 - 84%	25.50 (2.83)	33.70 (1.74)	0.01 (0.001)
10	Galvanized Metal	30	200	23 - 25	80 - 84%	2.46 (0.95)	1.02 (0.33)	0.01 (0.002)
10	Decorative Laminate	30	200	23 - 25	80 - 84%	9.29 (1.91)	4.47 (1.29)	0.01 (0.001)
10	Cellulose Insulation	30	200	23 - 25	80 - 84%	4.70 (0.71)	4.24 (1.42)	0.27 (0.07)
10	Particle Board	30	200	23 - 25	80 - 84%	3.52 (1.45)	4.52 (1.41)	0.21 (0.11)
10	Industrial Carpet	30	200	23 - 25	80 - 84%	30.22 (2.84)	26.33 (8.15)	0.62 (0.69)

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 4-12. Geometric Mean Percent Reduction of Ricin Cytotoxicity with ClO_2 Fumigation at Various CTs

Trial	CT, ppmv-hr	[ClO_2] ppmv	Contact Time, min	Material	Mean % Reduction (95% Confidence Interval) and p-Value*
9	500	1500	20	Glass (small)	**99.84% (99.76% , 99.90%) p=<0.0001**
9	500	1500	20	Painted Concrete	**99.96% (99.95% , 99.97%) p=<0.0001**
9	500	1500	20	Galvanized Metal	**99.46% (97.51% , 99.88%) p=<0.0001**
9	500	1500	20	Decorative Laminate	**99.13% (97.79% , 99.66%) p=<0.0001**
9	500	1500	20	Cellulose Insulation	**92.74% (81.69% , 97.12%) p=0.0003**
9	500	1500	20	Particle Board	**99.46% (99.29% , 99.58%) p=<0.0001**
9	500	1500	20	Industrial Carpet	**99.61% (99.43% , 99.74%) p=<0.0001**
10	100	200	30	Glass (small)	**99.75% (99.67% , 99.81%) p=<0.0001**
10	100	200	30	Painted Concrete	**99.97% (99.97% , 99.97%) p=<0.0001**
10	100	200	30	Galvanized Metal	**98.49% (97.92% , 98.91%) p=<0.0001**
10	100	200	30	Decorative Laminate	**99.69% (99.59% , 99.76%) p=<0.0001**
10	100	200	30	Cellulose Insulation	**93.42% (89.26% , 95.97%) p=<0.0001**
10	100	200	30	Particle Board	**95.81% (92.17% , 97.75%) p=<0.0001**
10	100	200	30	Industrial Carpet	**98.31% (95.23% , 99.40%) p=<0.0001**

*Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. This result was transformed to percentage. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.
Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

4.1.8 ClO_2 Fumigation of Vaccinia Virus at Two CTs

Trials 13 and 14, shown in Tables 4-13 and 4-14, evaluated the efficacy of ClO_2 fumigation against vaccinia virus at two CT values. Trial 13 used a fumigation CT of 500 ppmv-hr (1500 ppmv for 20 min) against vaccinia virus applied to seven types of coupons. Trial 14 used a fumigation CT of 125 ppmv-hr (250 ppmv for 30 min) against vaccinia virus applied to six types of coupons. During fumigation the temperature was maintained at 24 ± 2 °C and the RH was controlled at 80 ± 5% (full scale). The ClO_2 fumigation levels of 500 ppmv-hr and 125 ppmv-hr each resulted in no plaque-forming virus being recovered from any coupons. For coupons with high recovery efficiencies, the complete inactivation of viable virus equated to >6 log inactivation after the fumigation.

The vaccinia results exhibited complete inactivation for the decontaminated coupons within every test, with each test result compared to its respective controls yielding a statistically significant mean log reduction. Due to the complete decontamination observed in all tests, no statistical comparison can be made between the two concentration and time conditions for each material.

4.1.9 Summary of Findings from the ClO_2 Fumigation Investigation

- ClO_2 fumigation was efficacious for the decontamination of *B. anthracis* Ames spores (and surrogates *B. anthracis* NNR1Δ1 and *B. subtilis*), ricin, and vaccinia virus from all of a broad range of indoor building materials tested.
- A CT of 1000 ppmv-hr of ClO_2 resulted in a >7 log reduction in viable *B. anthracis* Ames spores (no CFU detected) from six of the seven building materials tested (all except compressed cellulose insulation) at 24 - 27 °C and >80% RH.
- Compressed cellulose insulation required 12,000 ppmv-hr at 24 - 27 °C and >80% RH or 9,000 ppmv-hr at elevated temperature of 30 - 32 °C and 74 - 79% RH for a >7 log reduction in *B. anthracis* Ames spores (no CFU detected). This limited investigation suggests that raising the temperature may increase efficacy. While the temperature impact was statistically significant, further testing is necessary to draw a firm conclusion.
- High RH (82 - 84%) appears to be more efficacious than lower RH (75 - 77%).
- Whether a CT of 2,250 was generated at lower concentrations for a longer time (750 ppmv, 3 hr) or a higher concentration for a shorter time (3000 ppmv, 0.75 hr) did not change the observed >7 log reduction efficacy against *B. anthracis* Ames on painted concrete; the extrapolate of this result to other concentration – contact time combinations is not clear from this work.
- A CT of 500 ppmv-hr of ClO_2 resulted in a geometric mean reduction of ricin of >99.1% from all materials tested except cellulose insulation which exhibited a 92.7% reduction.
- A CT of 125 ppmv-hr of ClO_2 resulted in no vaccinia virus being recovered from any coupon of any of the seven materials tested.

Table 4-13. Vaccinia PFU with and without ClO_2 Fumigation at Various CTs

Trial	Material	Contact Time, min	[ClO_2], ppmv	Temperature, °C	RH, %	T0 Control Mean PFU (SD)	20 or 30 min Control Mean PFU (SD)	ClO_2 Test Mean PFU*
13	Glass (small)	20	1500	22.7 - 23.7	80 - 83	3.93×10^6 (4.26×10^5)	2.29×10^6 (1.05×10^6)	0
13	Painted Concrete	20	1500	22.7 - 23.7	80 - 83	7.61×10^6 (4.42×10^5)	5.67×10^6 (1.74×10^6)	0
13	Galvanized Metal	20	1500	22.7 - 23.7	80 - 83	5.19×10^7 (1.45×10^7)	1.07×10^7 (3.12×10^6)	0
13	Decorative Laminate	20	1500	22.7 - 23.7	80 - 83	3.97×10^7 (1.02×10^7)	2.09×10^7 (8.44×10^6)	0
13	Cellulose Insulation	20	1500	22.7 - 23.7	80 - 83	7.34×10^5 (1.44×10^5)	8.41×10^5 (2.51×10^5)	0
13	Particle Board	20	1500	22.7 - 23.7	80 - 83	1.75×10^3 (1.69×10^3)	1.81×10^3 (2.07×10^3)	0
13	Industrial Carpet	20	1500	22.7 - 23.7	80 - 83	4.22×105 (3.13×10^5)	2.25×10^5 7.91×10^4)	0
14	Glass (small)	30	250	22.1 - 22.4	75 - 78	3.73×10^4 (6.65×10^3)	3.22×10^4 (3.12×10^3)	0
14	Painted Concrete	30	250	22.1 - 22.4	75 - 78	4.51×10^5 (4.63×10^4)	3.25×10^5 (5.45×10^4)	0
14	Galvanized Metal	30	250	22.1 - 22.4	75 - 78	3.85×10^5 (1.62×10^5)	2.95×10^5 (9.11×10^4)	0
14	Decorative Laminate	30	250	22.1 - 22.4	75 - 78	3.86×10^5 (7.54×10^4)	2.32×10^5 (1.53×10^4)	0
14	Cellulose Insulation	30	250	22.1 - 22.4	75 - 78	5.28×10^5 (5.43×10^4)	3.67×10^5 (3.12×10^5)	0
14	Particle Board	30	250	22.1 - 22.4	75 - 78	1.07×10^5 (1.31×10^4)	1.10×10^5 (1.30×10^4)	0
14	Industrial Carpet	30	250	22.1 - 22.4	75 - 78	2.95×10^5 (1.43×10^5)	2.98×10^5 (8.74×10^4)	0

***** SD not calculated when no viruses are recovered from any test coupon. Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 4-14. Mean Log Reduction of Vaccinia PFU with ClO_2 Fumigation at Various CTs

Trial	CT, ppmv-hr	[ClO_2], ppmv	Contact Time, min	Material	Mean Log Reduction (95% Confidence Interval) and p-Value[*H]
13	125	250	30	Glass (small)	**>4.51 (5/5) p=0.0135**
13	125	250	30	Painted Concrete	**>5.51,(5/5) p=0.0135**
13	125	250	30	Galvanized Metal	**>5.34 (5/5) p=0.0135**
13	125	250	30	Decorative Laminate	**>5.36 (5/5) p=0.0135**
13	125	250	30	Cellulose Insulation	**>5.43 (5/5) p=0.0135**
13	125	250	30	Particle Board	**>5.04 (5/5) p=0.0135**
13	125	250	30	Industrial Carpet	**>5.45 (5/5) p=0.0135**
14	500	1500	20	Glass (small)	**>6.32 (5/5) p=0.0135**
14	500	1500	20	Painted Concrete	**>6.74 (5/5) p=0.0135**
14	500	1500	20	Galvanized Metal	**>7.02 (5/5) p=0.0135**
14	500	1500	20	Decorative Laminate	**>7.30 (5/5) p=0.0135**
14	500	1500	20	Cellulose Insulation	**>5.91 (5/5) p=0.0135**
14	500	1500	20	Particle Board	**>2.32 (5/5) p=0.0135**
14	500	1500	20	Industrial Carpet	**>5.33 (5/5) p=0.0135**

[*]Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.

[H]One or more treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one PFU substituted for all zero recovery coupon values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicates statistically significantly greater reduction in the treated group than in the controls.

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

4.2 MeBr Fumigation Results

4.2.1 Results for MeBr Fumigation of B. anthracis on Glass and Ceiling Tile at Various CT Values (36 °C)

The efficacy of MeBr against *B. anthracis* Ames spores on glass and ceiling tile was evaluated using the quantitative method at CT values ranging from 315 to 2250 mg/L-hr at 75% RH and 36 °C. (Results are reported in mg/L rather than ppmv to be consistent with the historical precedent in previous efficacy studies of reporting MeBr concentrations as mg/L or g/L.) The results of these tests are shown in Tables 4-15 and 4-16 and Figure 4-1. A sigmoid log reduction vs. CT curve was observed. Complete kill of spores (0 CFU; >6 log reduction) from glass and ceiling tile was observed at a CT value of 2520 mg/L-hr. Low within-trial variability and high between-trial variability was observed along the sloped portion of the CT curve. Because within-trial variability was low, the observed uncertainty along the slope may suggest an unknown and uncontrolled variable that impacts efficacy in the transition range from about 1 log to >6 log reduction in viable spores.

Figure 4-1 shows that, for a given CT, a low concentration (53 mg/L) and higher contact time was generally less efficacious than a higher concentration (105, 212, or 320 mg/L) with a lower contact time for decontamination of *B. anthracis* Ames spores from glass or ceiling tile coupons.

Table 4-16 shows that, for *B. anthracis* Ames spores, at CT values of 795 mg/L-hr or higher (75% RH and 36 °C) significant log reductions were consistently observed on glass and ceiling tile. At CT values ≥795 mg/L-hr and <1260 mg/L-hr the log reductions were small (1.07 – 3.67), but significant.

Table 4-15. Results of Varying MeBr CT (36 °C and 75% RH)

Trial	*B. anthracis* Ames	[MeBr] Range (mg/L), Contact Time, CT	Temperature Range, °C	RH Range, %	Control Mean CFU (SD)	Decon Mean CFU (SD)†
1	Glass (small), 1x 10^7 spores applied	103 – 117 mg/L 24 hr ~2520 mg/L-hr	36 - 38	73 - 80	3.05 x 10^6 (2.51 x 10^5)	0
1	Ceiling tile, 1x 10^8 spores applied	103 – 117 mg/L 24 hr ~2520 mg/L-hr	36 - 38	73 - 80	1.13 x 10^7 (1.46 x 10^6)	0
2	Glass (small), 1x 10^7 spores applied	99 – 114 mg/L 12 hr ~1260 mg/L-hr	35 - 37	70 - 76	2.93 x 10^5 (1.68 x 10^5)	0
2	Ceiling tile, 1x 10^8 spores applied	99 – 114 mg/L 12 hr ~1260 mg/L-hr	35 - 37	70 - 76	1.08 x 10^7 (2.37 x 10^6)	1.32 x 10^1 (1.81 x 10^1)
3	Glass (small), 1x 10^7 spores applied	98 – 110 mg/L 3 hr ~315 mg/L-hr	35 - 36	70 - 76	4.05 x 10^6 (4.52 x 10^5)	3.52 x 10^6 (4.73 x 10^5)
3	Ceiling tile, 1x 10^8 spores applied	98 – 110 mg/L 3 hr ~315 mg/L-hr	35 - 36	70 - 76	2.06 x 10^7 (9.34 x 10^6)	6.83 x 10^6 (3.55 x 10^6)
4	Glass (small), 1x 10^7 spores applied	103 – 109 mg/L 6 hr ~630 mg/L-hr	35 - 36	70 - 76	5.46 x 10^6 (1.58 x 10^6)	2.29 x 10^6 (9.54 x 10^5)
4	Ceiling tile, 1x 10^8 spores applied	103 – 109 mg/L 6 hr ~630 mg/L-hr	35 - 36	70 - 76	1.57 x 10^7 (6.90 x 10^6)	9.85 x 10^6 (2.22 x 10^6)
5	Glass (small), 1x 10^7 spores applied	102-110 mg/L 9 hr ~945 mg/L-hr	35 - 36	71 - 76	4.78 x 10^6 (5.06 x 10^5)	4.34 x 10^5 (1.97 x 10^5)
5	Ceiling tile, 1x 10^8 spores applied	102-110 mg/L 9 hr ~945 mg/L-hr	35 - 36	71 - 76	1.93 x 10^7 (3.97 x 10^6)	1.68 x 10^6 (7.00 x 10^5)
6	Glass (small), 1x 10^7 spores applied	201-216 mg/L 6 hr ~1272 mg/L-hr	35 - 37	72 - 79	4.39 x 10^6 (7.65 x 10^5)	3.13 x 10^4 (2.79 x 10^4)
6	Ceiling tile, 1x 10^8 spores applied	201-216 mg/L 6 hr ~1272 mg/L-hr	35 - 37	72 - 79	9.01 x 10^6 (9.32 x 10^5)	3.57 x 10^5 (8.87 x 10^4)
7	Glass (small), 1x 10^7 spores applied	199-218 mg/L 9 hr ~1908 mg/L-hr	35 - 37	72 - 75	4.04 x 10^6 (5.94 x 10^5)	0
7	Ceiling tile, 1x 10^8 spores applied	199-218 mg/L 9 hr ~1908 mg/L-hr	35 - 37	72 - 75	7.77 x 10^6 (7.86 x 10^5)	1.34 x 10^1 (3.00 x 10^1)

†SD not calculated when no spores are recovered from any test coupon.
Note [MeBr] is concentration of MeBr in the chamber atmosphere, in mg/L.

Table 4-15. Continued

Trial	*B. anthracis* Ames	[MeBr] Range (mg/L), Contact Time, CT	Temperature Range, oC	RH Range, %	Control Mean CFU (SD)	Decon Mean CFU (SD)*
8	Glass (small), 1x 10^7 spores applied	201-212 mg/L 7 hr ~1484 mg/L-hr	35 - 37	71 - 77	2.08 x 10^7 (7.54 x 10^6)	8.47 x 10^2 (1.14 x 10^2)
8	Ceiling tile, 1x 10^8 spores applied	201-212 mg/L 7 hr ~1484 mg/L-hr	35 - 37	71 - 77	5.15 x 10^7 (9.16 x 10^6)	3.39 x 10^4 (5.91 x 10^3)
9	Glass (small), 1x 10^7 spores applied	200-212 mg/L 7 hr ~1484 mg/L-hr	35 - 36	36 - 43	9.38 x 10^6 (5.64 x 10^5)	8.21 x 10^3 (8.53 x 10^2)
9	Ceiling tile, 1x 10^8 spores applied	200-212 mg/L 7 hr ~1484 mg/L-hr	35 - 36	36 - 43	3.02 x 10^7 (2.77 x 10^6)	7.25 x 10^4 (1.01 x 10^4)
10	Glass (small), 1x 10^7 spores applied	50-54 mg/L 15 hr ~795 mg/L-hr	35 - 37	70 - 75	6.37 x 10^6 (4.24 x 10^5)	1.90 x 10^5 (1.13 x 10^5)
10	Ceiling tile, 1x 10^8 spores applied	50-54 mg/L 15 hr ~795 mg/L-hr	35 - 37	70 - 75	5.07 x 10^7 (3.69 x 10^6)	2.03 x 10^6 (2.18 x 10^5)
11	Glass (small), 1x 10^7 spores applied	52-56 mg/L 18 hr ~954 mg/L-hr	35 - 37	71 - 77	5.92 x 10^6 (8.86 x 10^5)	2.33 x 10^3 (1.82 x 10^3)
11	Ceiling tile, 1x 10^8 spores applied	52-56 mg/L 18 hr ~954 mg/L-hr	35 - 37	71 - 77	3.16 x 10^7 (6.91 x 10^6)	3.98 x 10^5 (1.06 x 10^5)
12	Glass (small), 1x 10^7 spores applied	51-55 mg/L 24 hr ~1272 mg/L-hr	35 - 37	71 - 77	8.31 x 10^6 (1.26 x 10^6)	0
12	Ceiling tile, 1x 10^8 spores applied	51-55 mg/L 24 hr ~1272 mg/L-hr	35 - 37	71 - 77	5.22 x 10^7 (9.18 x 10^6)	2.95 x 10^3 (1.42 x 10^3)
13	Glass (small), 1x 10^7 spores applied	102-108 mg/L 12 hr ~1250 mg/L-hr	35 - 37	71 - 74	3.26 x 10^6 (4.55 x 10^5)	3.44 x 10^4 (7.42 x 10^3)
13	Ceiling tile, 1x 10^8 spores applied	102-108 mg/L 12 hr ~1250 mg/L-hr	35 - 37	71 - 74	9.73 x 10^6 (2.92 x 10^5)	3.50 x 10^5 (6.01 x 10^4)

[†]SD not calculated when no spores are recovered from any test coupon.
Note [MeBr] is concentration of MeBr in the chamber atmosphere, in mg/L.

Table 4-16. Analysis of Results of Varying MeBr CT (36 °C and 75% RH) for Mean Log Reduction of *B. anthracis* Ames

Trial	Nominal CT, mg/L-hr	Time, hr	Actual [MeBr], mg/L	Mean Log Reduction (95% Confidence Interval) and p-value*	
				Glass	Ceiling tile
3	315	3	98-110	Not significant	**0.54 (0.08, 1.00) p=0.0356**
4	630	6	103-109	**0.40 (0.15, 0.65) p=0.0094**	Not significant
10	795	15	50-54	**1.59 (1.32, 1.86) p=0.0002**	**1.40 (1.34, 1.46) p<0.0001**
5	945	9	102-110	**1.07 (0.89, 1.25) p<0.0001**	**1.09 (0.86, 1.31) p<0.0001**
11	954	18	52-56	**3.67 (2.95, 4.39)**	**1.91 (1.75, 2.06) p<0.0001**
14	1260	12	102-108	**0.64 (0.53, 0.75) p<0.0001**	**1.26 (1.04, 1.4675) P<0.0001**
13	1260	12	102 - 108	**1.98 (1.87, 2.09) p<0.0001**	**1.45 (1.37, 1.53) p<0.0001**
2	1260	12	99-114	**> 6.46 (5/5)[H] p=0.0135**	**> 6.42 (3/5)[H] P<0.0135**
6	1272	6	201-216	**2.49 (1.70, 3.27) p=0.0017**	**1.41 (1.29, 1.54) p<0.0001**
12	1272	24	51-55	**> 6.92 (5/5)[H] p=0.0135**	**4.33 (3.93, 4.74) p<0.0001**
8	1484	7	201-212	**4.36 (4.16, 4.56) p<0.0001**	**3.18 (3.07, 3.29) p<0.0001**
17	1575	15	102-109	**>6.50 (5/5)[H] p=0.0135**	**4.73 (3.98, 5.48) P<0.0001**
16	1890	18	99-105	**>6.67 (5/5)[H] p=0.0135**	**>7.11 (5/5)[H] p=0.0135**
15	1908	9	198-210	**>5.75 (4/5)[H] p=0.0135**	**2.72 (1.92, 3.53) p=0.0013**
7	1908	9	199-218	**> 6.60 (5/5)[H] p=0.0135**	**> 6.52 (4/5)[H] p=0.0135**
1	2520	24	103 - 117	**> 6.48 (5/5)[H] p=0.0135**	**> 7.05 (5/5) [H] p=0.0135**

*Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.

[H] One or more treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one CFU substituted for all zero recovery coupons values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicates statistically significantly greater reduction in the treated group than in the controls.

Note [MeBr] is concentration of MeBr in the chamber atmosphere, in mg/L.

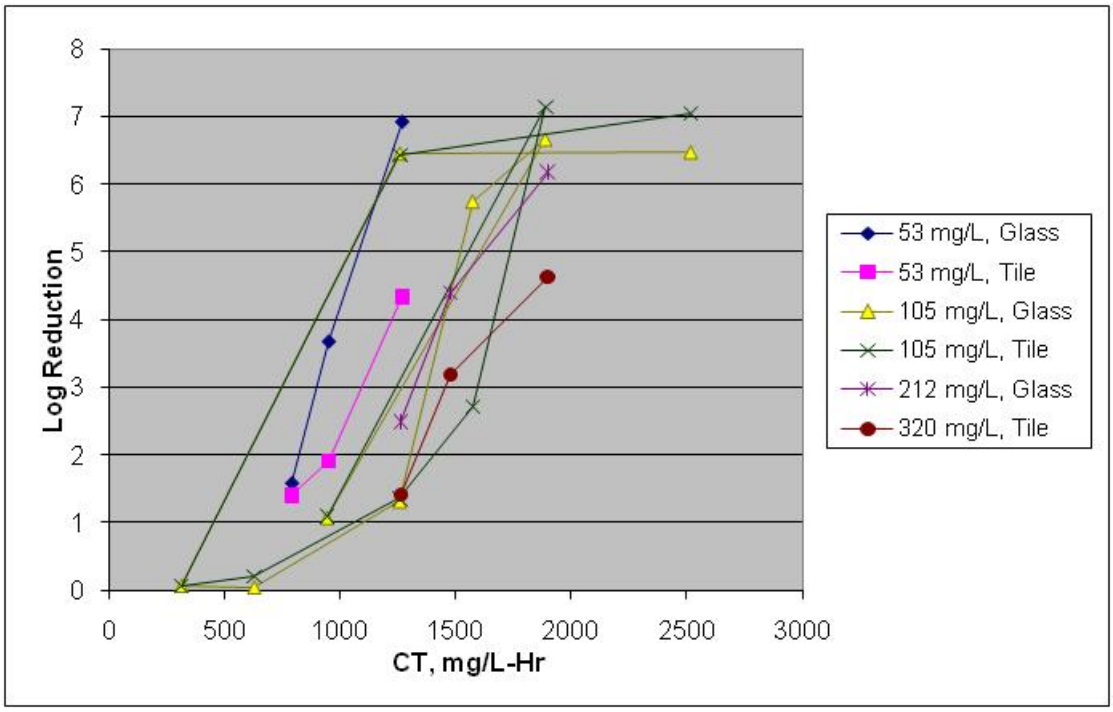


Figure 4-1. Mean *B. anthracis* log reduction from glass and ceiling tile vs. CT at various concentrations of MeBr.

4.2.2 Results for MeBr Fumigation of B. anthracis on Various Materials at Various CT Values (36 °C, 75% RH)

The efficacy of MeBr fumigation against *B. anthracis* Ames spores on five additional types of materials was evaluated using the quantitative method at four contact times. The fumigation was performed at 75% RH and 36 °C at 105 or 212 mg/L MeBr. The results of these tests are shown in Table 4-17 and Figure 4-2. For all materials, little or no significant log reduction (<2 log) was observed at CT of 1260 mg/L-hr. Greater than 6 log reduction was observed for *B. anthracis* on all materials except cellulose at a CT of 1900 (1890-1908) mg/L, whether by 18-hr contact with 105 mg/L MeBr or 9 hr contact with 212 mg/L MeBr.

As shown in Figure 4-3, very similar results were observed in log reduction at a CT of about ~1900 mg/L-hr whether 105 mg/L for 18 hr or 212 mg/L for 9 hr was used for the fumigation. For cellulose, no significant difference was found between the two CTs. For tile, the lower concentration, longer dwell time gave statistically significantly better mean log reduction (at least 2.37 log more) than the higher concentration, shorter dwell time. However, this result appears to reflect trial to trial variability rather than a difference attributable to the approach used to generate the CT. For all other materials (glass, painted concrete, laminate, galvanized metal, and carpet), there was complete kill for at least one coupon of each of the two CT values; no statistical comparison could be performed.

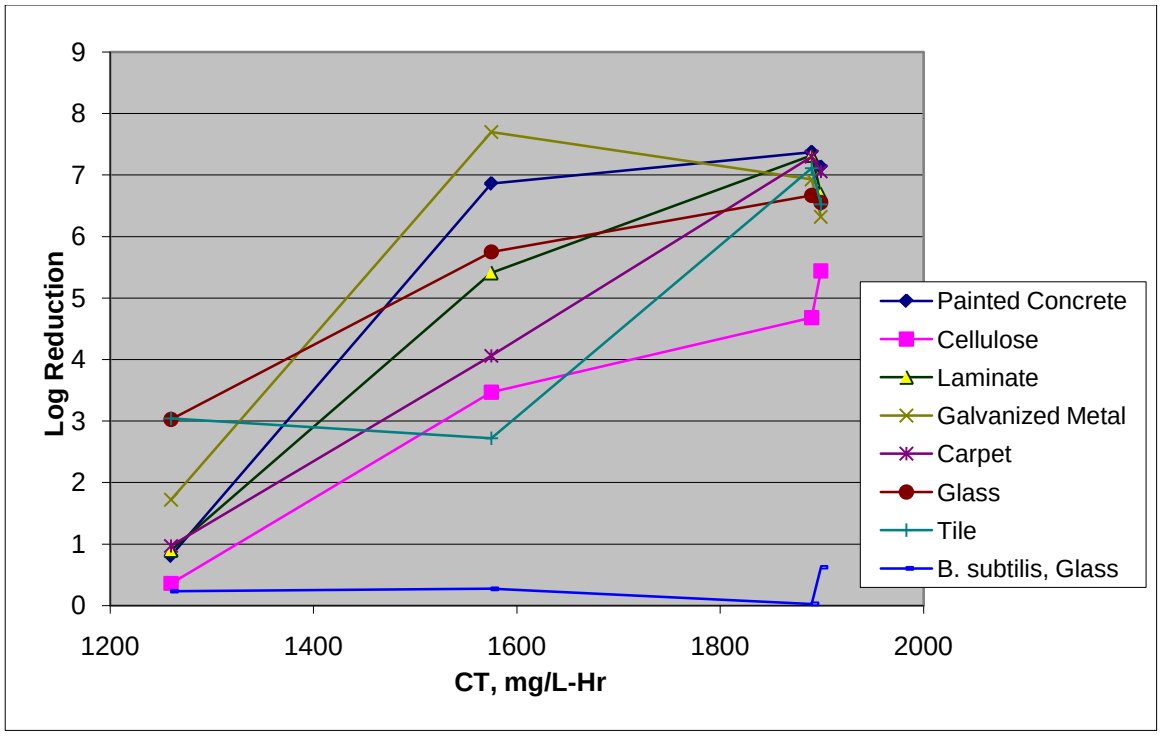


Figure 4-2. Mean *B. anthracis* (*B. subtilis* on glass) log reduction from various materials vs. CT.

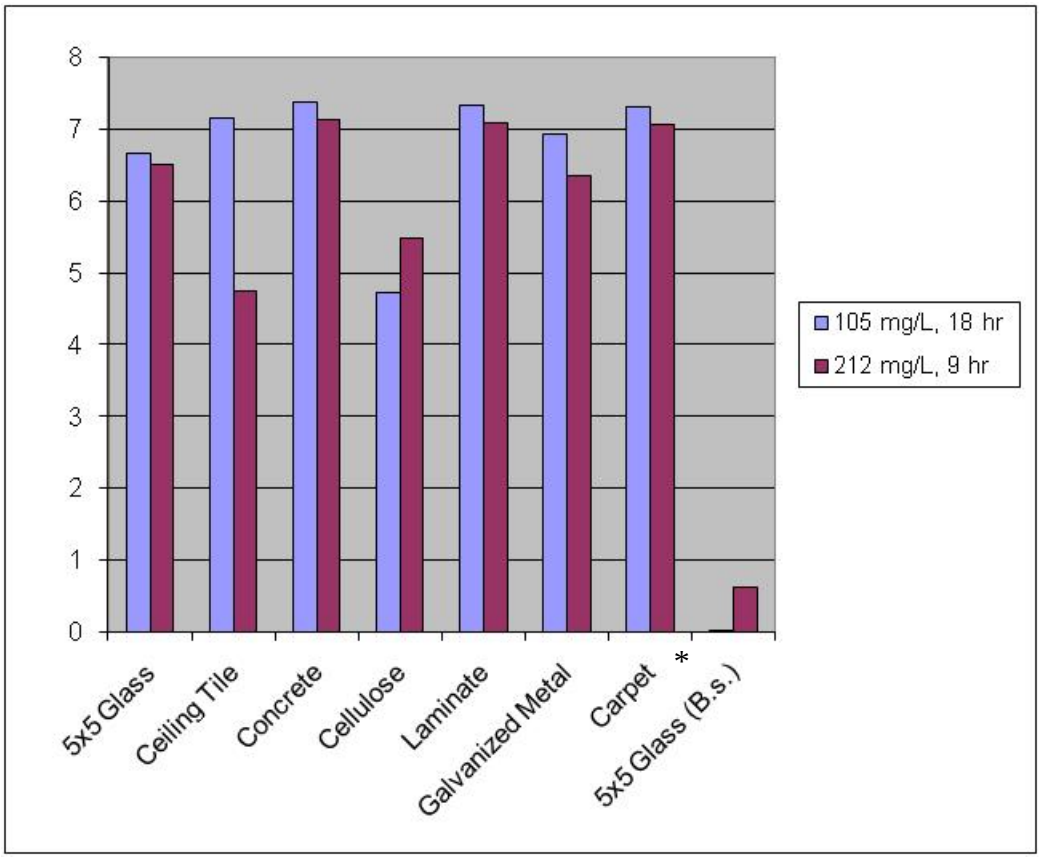
Table 4- 17. Results of Contact Time Series of MeBr Fumigation (36 °C and 75% RH) of *B. anthracis* Spores on Various Building Materials

Trial	CT, mg/L-hr	[MeBr] mg/L	Contact Time, hr	Mean Log Reduction (95% Confidence Interval) and p-Value*				
				Painted Concrete	Cellulose Insulation	Decorative Laminate	Galvanized Metal	Indust. Carpet
14	1260	102-108	12	0.81 (0.65, 0.96) p<0.0001	Not significant	0.90 (0.71, 1.28) p<0.0001	1.72 (1.16, 2.29) p=0.0018	0.97 (0.63, 1.31) p=0.0004
17	1575	102-109	15	>6.86 (3/5)[H] p=0.0135	3.47 (3.11, 3.82) p<0.0001	>5.41 (1/5)[H] p=0.0135	>7.6983 (5/5)[H] p=0.0135	4.06 (3.39, 4.74) p<0.0001
16	1890	99-105	18	>7.37 (4/5)[H] p=0.0135	4.68 (3.90, 5.47) p<0.0001	>7.32 (5/5)[H] p=0.0135	>6.93 (3/5)[H] p=0.0135	>7.30 (4/5)[H] p=0.0135
15	1908	198-210	9	>7.13 (4/5)[H] p=0.0135	>5.44 (1/5)[H] p=0.0135	>6.69 (3/5)[H] p=0.0135	>6.32 (3/5)[H] p=0.0135	>7.06 (4/5)[H] p= 0.0135

*Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons. Confidence intervals and p-values are from two sample t-tests comparing logs of control and treated coupons. Bolded results are statistically significant at 0.05 level.

[H] One or more treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported "*>x*" value is the mean log reduction with one CFU substituted for all zero recovery coupons values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicates statistically significantly greater reduction in the treated group than in the controls.

Note [MeBr] is concentration of MeBr in the chamber atmosphere, in mg/L.



* B.s. refers to *B. subtilis*

Figure 4-3. Mean *B. anthracis* log reduction from various materials (~1900 mg/L-hr).

4.2.3 Results for MeBr Fumigation of B. anthracis on Various Materials at 2 CT Values (25 °C and 36 °C, 75% RH)

Two trials were performed to evaluate whether lower temperature would significantly lower the efficacy of MeBr fumigation against *B. anthracis* Ames spores on eight types of materials. Efficacy was evaluated using the quantitative method at two contact times, i.e., 9 and 24 hr. The fumigation was performed at 75% RH and at the lower temperature of 25 °C at 212 mg/L MeBr. The results of these tests are shown in Table 4-18 and Figure 4-4. (Testing results at 36 °C are included for comparison.)

Except for carpet, the decontamination efficacy was much lower at 25 °C than at 36 °C. For all materials except carpet, low levels of log reduction (<4 log) were observed at a CT of 1908 mg/L-hr at the low temperature of 25 °C. In contrast, log reductions at the higher temperature of 36 °C were >5.4 for all materials. Except for cellulose and tile, the log reductions at the higher temperature were >6.3.

Greater than a 6 log reduction was observed for *B. anthracis* on all materials except ceiling tile at a CT of 5088 mg/L generated with a 24-hr contact at 212 mg/L MeBr.

Table 4-18. Results of 9 hr and 24 hr Contact Time of MeBr Fumigation (75% RH) of *B. anthracis* on Various Building Materials at a Target of 320 mg/L

Trial	CT, mg/L-hr	Temp., °C	Mean Log Reduction (SD)*							
			Painted Concrete	Cellulose Insulation	Decor. Laminate	Galv. Metal	Indust. Carpet	Glass	Ceiling Tile	Wood
18	1908 (212 mg/L, 9 hr)	25	2.20 (0.05)	2.33 (0.28)	>7.61 (0.00)	2.15 (0.12)	>7.71 (0.00)	2.55 (0.08)	2.85 (0.02)	3.52 (0.54)
15	1908 (212 mg/L, 9 hr)	36	>7.13 (1.38)	>5.47 (1.24)	>6.69 (1.24)	>6.35 (1.85)	>7.06 (1.28)	>6.51 (0.00)	4.74 (0.72)	NA
19	5088 (212 mg/L, 24 hr)	25	>7.92 (0.00)	6.02 (1.40)	>7.64 (0.00)	>7.61 (0.00)	>7.78 (0.00)	>7.70 (0.00)	4.55 (0.35)	7.64 (0.00)

*Mean log reduction is mean of logs of control coupons minus mean of logs of treated coupons.

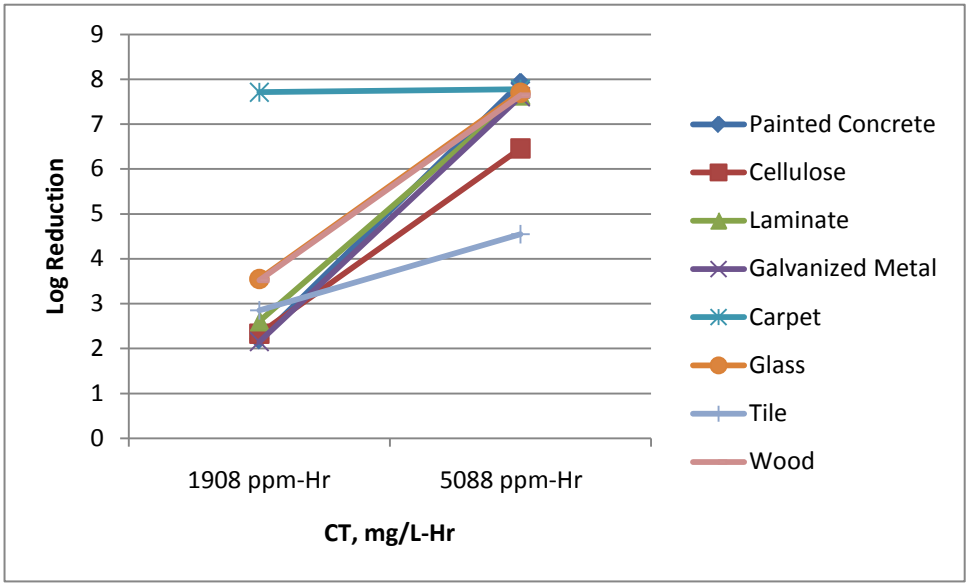


Figure 4-4. Mean *B. anthracis* log reduction from various materials vs. CT (25 °C, 75% RH, [MeBr] = 212 mg/L, contact times = 9 and 24 hr).

4.2.4 Results for MeBr Fumigation of B. subtilis on Glass at Various CT Values

B. subtilis was prepared and inoculated onto the small glass coupons in a manner identical to that used for *B. anthracis* and included in all of the test conditions.

As shown in Figure 4-5 and in Table 4-19, significant differences were observed between decontamination efficacy of MeBr fumigation against *B. anthracis* Ames and *B. subtilis* on glass. The difference in efficacy was very large (>5.48 log reduction difference) in the CT range of 1260 – 2880 mg/L-hr.

Table 4-19. Difference in Log Reduction of *B. anthracis* Ames and *B. subtilis* on Glass at Various CTs

CT, mg/L-hr	Bacterium	Difference in Mean Log Reduction and p-Value* Glass
1260	***B. anthracis* Ames vs. *B. subtilis***	**0.41 (0.25, 0.57) P<0.0001**
1575	***B. anthracis* Ames vs. *B. subtilis***	**>5.48 (4/5)[H] p=0.047**
1890	***B. anthracis* Ames vs. *B. subtilis***	**>6.64 (5/5)[H] p=0.047**
1890	***B. anthracis* Ames vs. *B. subtilis***	**>5.89 (5/5)[H] p=0.047**

*Difference in mean efficacy of paired test conditions. Confidence intervals and p-values are from two sample t-tests comparing mean efficacy results from two test conditions. Bolded results are statistically significant at 0.05 level.

[H] One or more treated coupons had no recovered agent (the exact number is shown in parentheses as test coupons with no growth/total test coupons). The reported ">x" value is the mean log reduction with one CFU substituted for all zero recovery coupons values to permit calculation of the log. For these trials, the test of statistical significance is a nonparametric Kolmogorov-Smirnov test where a p-value less than 0.05 indicates statistically significantly greater reduction in the treated group than in the controls.

As shown in Figure 4-5, little or no efficacy of MeBr against *B. subtilis* on glass was observed in the CT range or 1260-1908 mg/L-hr. In contrast, a high level of efficacy was observed against *B. anthracis* Ames on glass at a CT of 1575 or greater CT with no viable spores observed at CTs of 1890 mg/L-hr (105 mg/L for 18 hr) or 1908 mg/L-hr (212 mg/L for 9 hr).

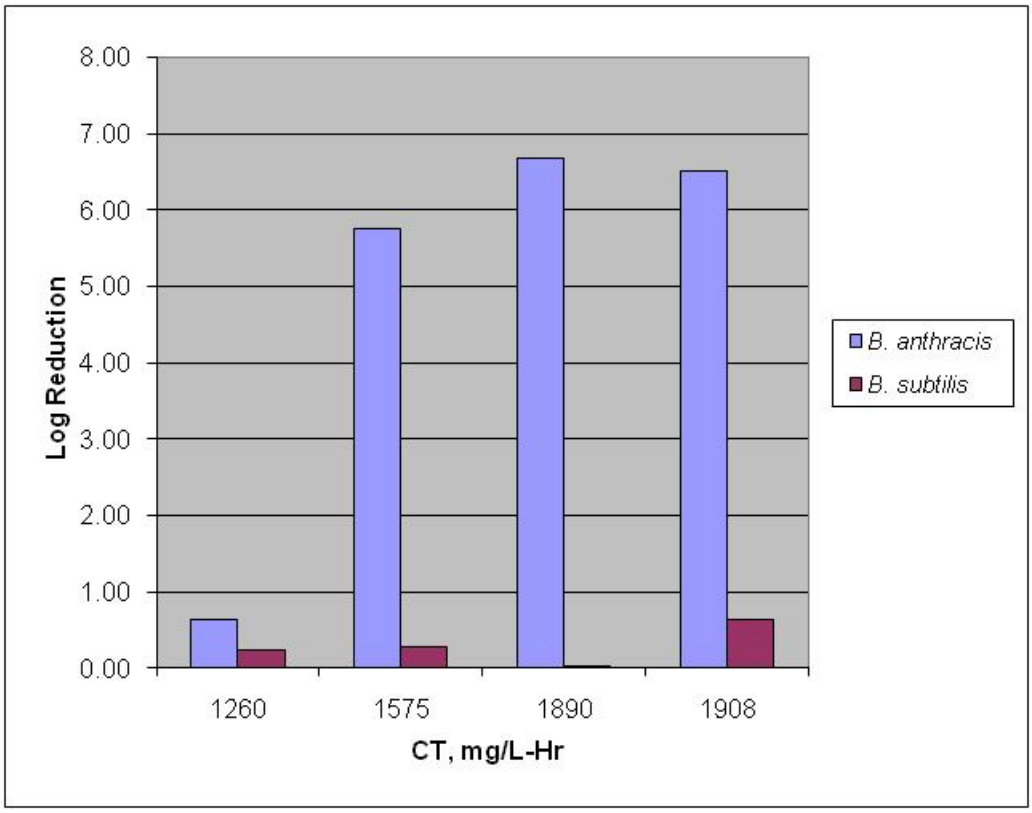


Figure 4-5. Mean log reduction of *B. anthracis* on various materials and *B. subtilis* spores on glass at various CTs.

4.2.5 Results for MeBr Fumigation of B. anthracis on Glass and Ceiling Tile at Alternate RH Conditions

A trial was performed to evaluate whether, at a given CT, efficacy would be different at a low RH (40%) during fumigation compared to a higher RH (75%) during fumigation. The results, shown in Table 4-20, indicated that there was a significant log reduction observed (compared to the controls) using MeBr at both RH conditions. The results also show that efficacy was higher at 75% RH than at 40% RH. However, as noted above, high between-trial variability was observed in the transition region of the CT curve between the low kill to complete kill. Because this was a single run with a relatively small, but significant, difference between the log reductions observed at the two RH conditions, no conclusions are conclusively drawn.

Table 4-20. Significance of Differences in Log Reduction Arising from Percent RH at 212 mg/L MeBr and 7-Hr Contact Time at 37 °C

Trial	CT	RH	Mean Log Reduction (SD)	
			Glass	Ceiling Tile
8	1484	75%	4.39 (0.06)	3.19 (0.08)
9	1484	40%	3.06 (0.04)	2.62 (0.06)
Statistical significance of difference between 75% RH and 40% RH			p< 0.0001	p< 0.0001

4.2.6 Results for MeBr Fumigation of B. subtilis on Suture Loops at Various CT Values

Suture loops, prepared and inoculated with *B. anthracis* spores as described for *B. subtilis* spores in AOAC method 966.04,[13] were included in the fumigation tests. The use of the qualitative suture loop test was included to provide a point of comparison between results from this investigation using *B. anthracis* spores and the results from AOAC 966.04 qualitative testing by others using *B. subtilis* spores as a surrogate.

After fumigation for a specific contact time, suture loops were placed into TSB and incubated for seven days. The tubes containing the inoculated suture loops were checked for cloudiness, indicating growth, on days one and seven. As shown in Table 4-21, at a CT of 1260 mg/L-hr (105 mg/L x 12 hr) the inoculated suture loops all resulted in cloudy media indicating growth of viable spores after fumigation. In contrast, after inoculated suture loops were exposed to MeBr at a CT of 1575 mg/L-hr (105 mg/L x 15 hr) or greater, no cloudiness was observed in the incubated medium consistent with complete kill of the spores. Positive and negative controls exhibited expected growth and no growth, respectively.

Table 4- 21. Qualitative Spore Viability Test

[MeBr]	105 mg/L			212 mg/L
Contact Time	12 hr	15 hr	18 hr	9 hr
Suture Loop Positive Control (n=5)	+	+	+	+
Suture Loop Decontamination Test Coupons (n=5)	+	-	-	-
Positive Control	+	+	+	+
Negative Control	-	-	-	-

"-" indicates that all coupons at the specified time and treatment exhibited no growth in TSB.
"+" indicates that all three coupons at the specified time and treatment exhibited growth in TSB.
Note [MeBr] is concentration of MeBr in the chamber atmosphere, in mg/L.

In Table 4-22, the log reduction in recovered spores from glass and ceiling tile after decontamination at a given CT is shown, along with the corresponding number of silk suture loop carriers that were positive for growth of *B. anthracis* Ames spores after the decontamination. The qualitative results from the suture loops and the log reduction from both glass and ceiling tile showed more growth of spores at the lower CT and less growth at the higher CT. The log reduction from glass is 5.75 or greater when no bacterial growth is observed from any of the five replicate silk suture loops included in the test. However, ceiling tile exhibited a log reduction as low as 2.73 when all silk suture loops were exhibiting no growth. Suture loop decontamination efficacy appeared to be more similar to the decontamination of nonporous than porous materials; i.e., silk suture loops appeared to be easier to decontaminate than the porous material.

Table 4-22. Comparison of Results for Log Reduction and Qualitative Spore Viability Test at Various CT Conditions

	Log Reduction		Silk Suture Loops Exhibiting No
CT, mg/L	Glass	Ceiling Tile	Growth/ Total
1260	0.64	1.26	5/5
1575	5.75	2.73	0/5
1890	6.67	7.15	0/5
1908	6.51	4.74	0/5

4.2.7 Results for MeBr Fumigation of B. atrophaeus on Stainless Steel in Tyvek® Packaging at Various CT Values

For all MeBr fumigation trials, BIs (*B. atrophaeus* on stainless steel in Tyvek® packaging [Apex Labs]) were included. At all CTs investigated, viable *B. atrophaeus* spores were detected in the qualitative viability test; fumigation did not kill all of the *B. atrophaeus* spores inside the Tyvek® packaging.

4.2.8 Summary of Findings from the MeBr Investigation

- MeBr fumigation was efficacious for the decontamination of *B. anthracis* Ames from all of a broad range of indoor building materials tested.
- A CT of 1890 mg/L-hr resulted in a >6 log reduction in viable *B. anthracis* Ames spores from all building materials except the compressed cellulose insulation and, in one trial only, from ceiling tile.
- Whether a given CT was generated at lower concentrations for a longer time (105 mg/L for 18 hr) or a higher concentration for a shorter time (212 mg/L – 9 hr) did not appear to impact the efficacy against *B. anthracis*; this result may not be generalized to lower concentrations of MeBr (53 mg/L) or to other

concentration and contact time combinations based upon these data alone.

- The CT range that resulted in a 1 to 6 log reduction in viable *B. anthracis* Ames spores exhibited little or no efficacy against *B. subtilis* on glass; *B. subtilis* on glass appears to be a conservative surrogate for *B. anthracis* Ames in MeBr fumigation.
- A small, but significant, difference in efficacy between 75% and 40% RH was observed. Because of the small difference in a single trial, no conclusions are drawn. However, even if the difference was caused by RH, the difference was so small as to have limited practical significance.
- When tested at a higher temperature (36 ^{o}C vs. 25 ^{o}C), mean log reductions in *B. anthracis* were considerably higher on the painted concrete, cellulose, galvanized metal, glass, and ceiling tile (CT of 1908 mg/L-hr MeBr).
- No viable spores were detected on silk suture loops after exposure to a MeBr CT of 1575 mg/L-hr. These results suggest that *B. anthracis* spores on silk suture loops are easier to kill than on any other material tested except galvanized metal.
- The qualitative results from the suture loops and the log reduction from both glass and ceiling tile show more growth of spores at the lower CT and less growth at the higher CT. The log reduction from glass is 5.75 or greater when no bacterial growth is observed from any of the five replicate silk suture loops included in the test.

4.3 HP Fumigation

4.3.1 Results of Varying HP CT at 22 °C

The efficacies of HP fumigation against *B. anthracis* Ames spores on nine materials at varying CT values are shown in Table 4-23 and Figures 4-6 and 4-7. No viable *B. anthracis* spores were recovered from carpet, painted concrete, glass, aluminum, keyboard, laminate, ductwork, and ceiling tile at exposures of 120 min to the 500 ppmv HP fumigation cycle. Decontamination of wood was more difficult than other materials: *B. anthracis* spores were still recovered from wood after 120-min exposure to the 500 ppmv HP fumigation cycle. No viable *B. anthracis* spores were recovered from wood at an exposure of 240 min to the 500 ppmv HP fumigation cycle.

With the 200-250 ppmv HP fumigation cycle, shown in Table 4-24, *B. anthracis* spores were not recovered from laminate, ductwork, painted concrete, glass, and ceiling tile after 120 min of exposure, but *B. anthracis* spores were recovered from carpet and wood after the entire 240-min fumigation cycle. For perspective, VHP® sterilant when used with a STERIS VHP® generator for sterilization of exposed pre-cleaned dry porous and non-porous surfaces, specifies a sterilization phase with a

minimum of 250 ppm[v] of VHP® sterilant for 90 min in sealed enclosures	up to 4,000 ft^3 [113,000 L].

Table 4-23. STERIS VHP® Fumigation Results for *B. anthracis* (500 ppmv)

Trial	Contact Time, min CT, ppmv-hr	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)* Positive Control†	Test Coupon‡¶	Mean Log Reduction*¶
500 ppmv Fumigation Cycle						
1	30	Finished aluminum	4.87×10^{8} §	$7.30 \pm 4.03 \times 10^{8}$ #	0	8.86 ± 0.00
	250	Computer keyboard keys	4.87×10^{8} §	$3.96 \pm 3.29 \times 10^{8}$	0	8.60 ± 0.00
		Industrial carpet	4.87×10^{8} §	$7.70 \pm 4.24 \times 10^{8}$ #	$2.73 \pm 4.06 \times 10^{5}$	4.31 ± 1.21
		Painted joint tape	4.87×10^{8} §	$5.42 \pm 1.72 \times 10^{8}$	0	8.73 ± 0.00
1	60	Finished aluminum	4.87×10^{8} §	$7.30 \pm 4.03 \times 10^{8}$ #	0	8.86 ± 0.00
	500	Computer keyboard keys	4.87×10^{8} §	$3.96 \pm 3.29 \times 10^{8}$	0	8.60 ± 0.00
		Industrial carpet	4.87×10^{8} §	$7.70 \pm 4.24 \times 10^{8}$ #	$1.85 \pm 2.56 \times 10^{4}$	6.70 ± 2.35
		Painted joint tape	4.87×10^{8} §	$5.42 \pm 1.72 \times 10^{8}$	0	8.73 ± 0.00
1	120	Finished aluminum	4.87×10^{8} §	$7.30 \pm 4.03 \times 10^{8}$ #	0	8.86 ± 0.00
	1000	Computer keyboard keys	4.87×10^{8} §	$3.96 \pm 3.29 \times 10^{8}$	0	8.60 ± 0.00
		Industrial carpet	4.87×10^{8} §	$7.70 \pm 4.24 \times 10^{8}$ #	0	8.89 ± 0.00
		Painted joint tape	4.87×10^{8} §	$5.42 \pm 1.72 \times 10^{8}$	0	8.73 ± 0.00
1	240	Finished aluminum	4.87×10^{8} §	$7.30 \pm 4.03 \times 10^{8}$ #	0	8.86 ± 0.00
	2000	Computer keyboard keys	4.87×10^{8} §	$3.96 \pm 3.29 \times 10^{8}$	0	8.60 ± 0.00
		Industrial carpet	4.87×10^{8} §	$7.70 \pm 4.24 \times 10^{8}$ #	0	8.89 ± 0.00
		Painted joint tape	4.87×10^{8} §	$5.42 \pm 1.72 \times 10^{8}$	0	8.73 ± 0.00
1	(Full cycle)	Finished aluminum	4.87×10^{8} §	$7.30 \pm 4.03 \times 10^{8}$ #	0	8.86 ± 0.00
	240	Computer keyboard keys	4.87×10^{8} §	$3.96 \pm 3.29 \times 10^{8}$	0	8.60 ± 0.00
	2000	Industrial carpet	4.87×10^{8} §	$7.70 \pm 4.24 \times 10^{8}$ #	0	8.89 ± 0.00
		Painted joint tape	4.87×10^{8} §	$5.42 \pm 1.72 \times 10^{8}$	0	8.73 ± 0.00
2a	30	Decorative Laminate	2.80×10^{8} §	$2.61 \pm 1.58 \times 10^{8}$	0	8.42 ± 0.00
	250	Galvanized metal ductwork	2.80×10^{8} §	$1.67 \pm 1.04 \times 10^{8}$	0	8.22 ± 0.00
		Industrial carpet	2.80×10^{8} §	$2.13 \pm 2.39 \times 10^{8}$	$1.74 \pm 1.41 \times 10^{5}$	3.44 ± 0.85
		Painted concrete block	2.80×10^{8} §	$2.26 \pm 1.60 \times 10^{8}$	$1.20 \pm 2.68 \times 10^{2}$	7.80 ± 1.24
2a	60	Decorative Laminate	2.80×10^{8} §	$2.61 \pm 1.58 \times 10^{8}$	0	8.42 ± 0.00
	500	Galvanized metal ductwork	2.80×10^{8} §	$1.67 \pm 1.04 \times 10^{8}$	0	8.22 ± 0.00
		Industrial carpet	2.80×10^{8} §	$2.13 \pm 2.39 \times 10^{8}$	$6.67 \pm 11.6 \times 10^{3}$	5.57 ± 1.69
		Painted concrete block	2.80×10^{8} §	$2.26 \pm 1.60 \times 10^{8}$	0	8.35 ± 0.00

* Data are expressed as mean ± SD of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
# T0 was lower than target recovery of ≥10% of spike amount. ¶ SD not calculated when no spores are recovered from any test coupon

Table 4-23. Continued

Trial	Contact Time, min CT, ppmv-hr	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*¶
				Positive Control†	Test Coupon‡¶	
2a	120	Decorative Laminate	2.80×10^{8} §	$2.61 \pm 1.58 \times 10^{8}$	0	8.42 ± 0.00
	1000	Galvanized metal ductwork	2.80×10^{8} §	$1.67 \pm 1.04 \times 10^{8}$	0	8.22 ± 0.00
		Industrial carpet	2.80×10^{8} §	$2.13 \pm 2.39 \times 10^{8}$	0	8.33 ± 0.00
		Painted concrete block	2.80×10^{8} §	$2.26 \pm 1.60 \times 10^{8}$	0	8.35 ± 0.00
2a	240	Decorative Laminate	2.80×10^{8} §	$2.61 \pm 1.58 \times 10^{8}$	0	8.42 ± 0.00
	2000	Galvanized metal ductwork	2.80×10^{8} §	$1.67 \pm 1.04 \times 10^{8}$	0	8.22 ± 0.00
		Industrial carpet	2.80×10^{8} §	$2.13 \pm 2.39 \times 10^{8}$	0	8.33 ± 0.00
		Painted concrete block	2.80×10^{8} §	$2.26 \pm 1.60 \times 10^{8}$	0	8.35 ± 0.00
2a	(Full cycle)	Decorative Laminate	2.80×10^{8} §	$2.61 \pm 1.58 \times 10^{8}$	0	8.42 ± 0.00
	240	Galvanized metal ductwork	2.80×10^{8} §	$1.67 \pm 1.04 \times 10^{8}$	0	8.22 ± 0.00
	2000	Industrial carpet	2.80×10^{8} §	$2.13 \pm 2.39 \times 10^{8}$	0	8.33 ± 0.00
		Painted concrete block	2.80×10^{8} §	$2.26 \pm 1.60 \times 10^{8}$	0	8.35 ± 0.00
3a	30	Pine wood	9.77×10^{6}	$5.47 \pm 1.57 \times 10^{5}$ #	$1.82 \pm 3.23 \times 10^{4}$	1.95 ± 0.66
	250	Plate glass	9.77×10^{6}	$8.18 \pm 10.5 \times 10^{6}$	$2.73 \pm 3.52 \times 10^{2}$	5.40 ± 1.42
		Ceiling tile	9.77×10^{6}	$7.49 \pm 1.40 \times 10^{5}$ #	0	5.87 ± 0.00
3a	60	Pine wood	9.77×10^{6}	$5.47 \pm 1.57 \times 10^{5}$ #	$4.51 \pm 4.41 \times 10^{3}$	2.97 ± 1.69
	500	Plate glass	9.77×10^{6}	$8.18 \pm 10.5 \times 10^{6}$	0	6.91 ± 0.00
		Ceiling tile	9.77×10^{6}	$7.49 \pm 1.40 \times 10^{5}$ #,	0	5.87 ± 0.00
3a	120	Pine wood	9.77×10^{6}	$5.47 \pm 1.57 \times 10^{5}$#	$7.19 \pm 8.28 \times 10^{2}$	3.51 ± 1.31
	1000	Plate glass	9.77×10^{6}	$8.18 \pm 10.5 \times 10^{6}$	0	6.91 ± 0.00
		Ceiling tile	9.77×10^{6}	$7.49 \pm 1.40 \times 10^{5}$ #	0	5.87 ± 0.00
3a	240	Pine wood	9.77×10^{6}	$5.47 \pm 1.57 \times 10^{5}$#	0	5.74 ± 0.00
	2000	Plate glass	9.77×10^{6}	$8.18 \pm 10.5 \times 10^{6}$	0	6.91 ± 0.00
		Ceiling tile	9.77×10^{6}	$7.49 \pm 1.40 \times 10^{5}$#	0	5.87 ± 0.00
3a	(Full cycle)	Pine wood	9.77×10^{6}	$5.47 \pm 1.57 \times 10^{5}$#	0	5.74 ± 0.00
	240 min	Plate glass	9.77×10^{6}	$8.18 \pm 10.5 \times 10^{6}$	0	6.91 ± 0.00
	2000	Ceiling tile	9.77×10^{6}	$7.49 \pm 1.40 \times 10^{5}$#	0	5.87 ± 0.00

* Data are expressed as mean ± SD of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
#T0 was lower than target recovery of ≥10% of spike amount.
¶SD not calculated when no spores are recovered from any test coupon

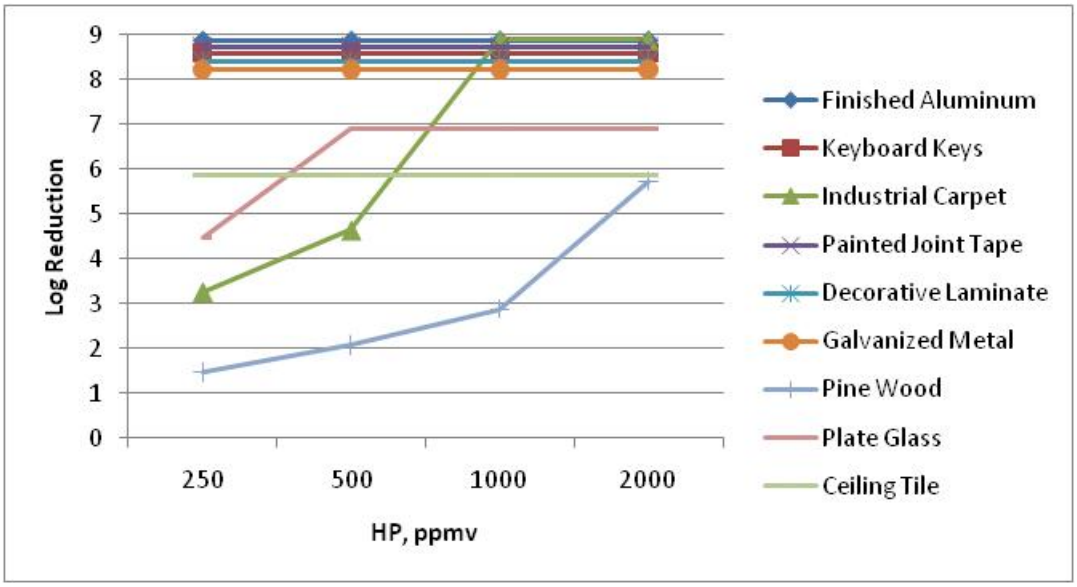


Figure 4-6. Mean *B. anthracis* spores log reduction from various materials vs. hydrogen peroxide CT (500 ppmv concentration) at 22 °C.

Table 4-24. STERIS VHP® Fumigation Results for *B. anthracis* Ames Spores (225 ppmv)

Trial	Contact Time, min CT, ppmv-hr	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)* Positive Control†	Test Coupon‡	Mean Log Reduction*
200-250 ppmv Fumigation Cycle						
2b	30	Decorative Laminate	6.93×10^{6} '	$3.22 \pm 0.50 \times 10^{6}$	$1.34 \pm 3.00 \times 10^{1}$	6.14 ± 0.82
	113	Galvanized metal ductwork	6.93×10^{6} '	$5.25 \pm 0.79 \times 10^{6}$	0	6.72 ± 0.00
		Industrial carpet	6.93×10^{6} '	$6.17 \pm 0.57 \times 10^{6}$	$3.35 \pm 0.74 \times 10^{5}$	1.27 ± 0.09
		Painted concrete block	6.93×10^{6} '	$6.73 \pm 0.70 \times 10^{6}$	$1.65 \pm 3.59 \times 10^{3}$	5.28 ± 1.63
2b	60	Decorative Laminate	6.93×10^{6} '	$3.22 \pm 0.50 \times 10^{6}$	0	6.51 ± 0.00
	225	Galvanized metal ductwork	6.93×10^{6} '	$5.25 \pm 0.79 \times 10^{6}$	0	6.72 ± 0.00
		Industrial carpet	6.93×10^{6} '	$6.17 \pm 0.57 \times 10^{6}$	$1.11 \pm 2.01 \times 10^{3}$	5.06 ± 1.67
		Painted concrete block	6.93×10^{6} '	$6.73 \pm 0.70 \times 10^{6}$	$6.60 \pm 14.8 \times 10^{0}$	6.52 ± 0.68
2b	120	Decorative Laminate	6.93×10^{6} '	$3.22 \pm 0.50 \times 10^{6}$	0	6.51 ± 0.00
	450	Galvanized metal ductwork	6.93×10^{6} '	$5.25 \pm 0.79 \times 10^{6}$	0	6.72 ± 0.00
		Industrial carpet	6.93×10^{6} '	$6.17 \pm 0.57 \times 10^{6}$	$2.00 \pm 4.47 \times 10^{1}$	6.39 ± 0.89
		Painted concrete block	6.93×10^{6} '	$6.73 \pm 0.70 \times 10^{6}$	0	6.83 ± 0.00
2b	240	Decorative Laminate	6.93×10^{6} '	$3.22 \pm 0.50 \times 10^{6}$	0	6.51 ± 0.00
	900	Galvanized metal ductwork	6.93×10^{6} '	$5.25 \pm 0.79 \times 10^{6}$	0	6.72 ± 0.00
		Industrial carpet	6.93×10^{6} '	$6.17 \pm 0.57 \times 10^{6}$	$2.00 \pm 2.99 \times 10^{1}$	6.12 ± 0.92
		Painted concrete block	6.93×10^{6} '	$6.73 \pm 0.70 \times 10^{6}$	0	6.83 ± 0.00
2b	(Full cycle)	Decorative Laminate	6.93×10^{6} '	$3.22 \pm 0.50 \times 10^{6}$	0	6.51 ± 0.00
	240	Galvanized metal ductwork	6.93×10^{6} '	$5.25 \pm 0.79 \times 10^{6}$	0	6.72 ± 0.00
	900	Industrial carpet	6.93×10^{6} '	$6.17 \pm 0.57 \times 10^{6}$	1.34 ± 3.00^{1}	6.42 ± 0.82
		Painted concrete block	6.93×10^{6} '	$6.73 \pm 0.70 \times 10^{6}$	0	6.83 ± 0.00

* Data are expressed as mean ± SD of five replicates. SD not calculated when no spores are recovered from any test coupon.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
' Application was lower than the target 7.5×10^{5} CFU/coupon.
T0 was lower than target recovery of ≥10% of spike amount.

Table 4-24. Continued

Trial	Contact Time, min CT, ppmv-hr	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		
				Positive Control†	Test Coupon‡	Mean Log Reduction*
3b	30	Pine wood	1.02×10^7	$3.59 \pm 0.63 \times 10^{5\#}$	$2.06 \pm 1.60 \times 10^4$	1.35 ± 0.35
	113	Plate glass	1.02×10^7	$3.53 \pm 1.68 \times 10^6$	$1.34 \pm 3.00 \times 10^1$	6.18 ± 0.82
		Ceiling tile	1.02×10^7	$7.62 \pm 2.18 \times 10^{5\#}$	$3.32 \pm 4.08 \times 10^1$	5.18 ± 0.98
3b	60	Pine wood	1.02×10^7	$3.59 \pm 0.63 \times 10^{5\#}$	$6.83 \pm 10.9 \times 10^3$	2.15 ± 0.70
	225	Plate glass	1.02×10^7	$3.53 \pm 1.68 \times 10^6$	$2.66 \pm 5.95 \times 10^1$	6.12 ± 0.95
		Ceiling tile	1.02×10^7	$7.62 \pm 2.18 \times 10^{5\#}$	$2.00 \pm 2.99 \times 10^1$	5.21 ± 0.92
3b	120	Pine wood	1.02×10^7	$3.59 \pm 0.63 \times 10^{5\#}$	$2.68 \pm 0.60 \times 10^3$	2.14 ± 0.10
	450	Plate glass	1.02×10^7	$3.53 \pm 1.68 \times 10^6$	0	6.55 ± 0.00
		Ceiling tile	1.02×10^7	$7.62 \pm 2.18 \times 10^{5\#}$	0	5.88 ± 0.00
3b	240	Pine wood	1.02×10^7	$3.59 \pm 0.63 \times 10^{5\#}$	$1.61 \pm 1.67 \times 10^3$	3.52 ± 1.87
	900	Plate glass	1.02×10^7	$3.53 \pm 1.68 \times 10^6$	0	6.55 ± 0.00
		Ceiling tile	1.02×10^7	$7.62 \pm 2.18 \times 10^{5\#}$	0	5.88 ± 0.00
3b	(Full cycle)	Pine wood	1.02×10^7	$3.59 \pm 0.63 \times 10^{5\#}$	$2.73 \pm 3.84 \times 10^4$	3.32 ± 2.45
	240	Plate glass	1.02×10^7	$3.53 \pm 1.68 \times 10^6$	0	6.55 ± 0.00
	900	Ceiling tile	1.02×10^7	$7.62 \pm 2.18 \times 10^{5\#}$	0	5.88 ± 0.00
D2	30	Particle board	1.11×10^8	$6.28 \pm 2.22 \times 10^7$	$1.14 \pm 1.07 \times 10^6$	1.86 ± 0.33
	113	Cellulose insulation	1.11×10^8	$2.05 \pm 0.97 \times 10^7$	$2.71 \pm 1.95 \times 10^6$	0.97 ± 0.32
D2	60	Particle board	1.11×10^8	$6.28 \pm 2.22 \times 10^7$	$1.90 \pm 2.81 \times 10^6$	1.86 ± 0.58
	225	Cellulose insulation	1.11×10^8	$2.05 \pm 0.97 \times 10^7$	$4.32 \pm 3.41 \times 10^5$	1.84 ± 0.48
D2	120	Particle board	1.11×10^8	$6.28 \pm 2.22 \times 10^7$	$6.72 \pm 2.37 \times 10^5$	2.00 ± 0.18
	450	Cellulose insulation	1.11×10^8	$2.05 \pm 0.97 \times 10^7$	$1.72 \pm 1.73 \times 10^5$	2.41 ± 0.74
D2	240	Particle board	1.11×10^8	$6.28 \pm 2.22 \times 10^7$	$8.92 \pm 2.47 \times 10^4$	2.86 ± 0.13
	900	Cellulose insulation	1.11×10^8	$2.05 \pm 0.97 \times 10^7$	$1.41 \pm 1.76 \times 10^4$	3.41 ± 0.53
D2	(Full cycle)	Particle board	1.11×10^8	$6.28 \pm 2.22 \times 10^7$	$2.24 \pm 1.28 \times 10^4$	3.52 ± 0.30
	240	Cellulose insulation	1.11×10^8	$2.05 \pm 0.97 \times 10^7$	$3.23 \pm 0.72 \times 10^3$	3.81 ± 0.10
	900					

* Data are expressed as mean ± SD of five replicates. SD not calculated when no spores are recovered from any test coupon.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
' Application was lower than the target 7.5×10^5 CFU/coupon.
T0 was lower than target recovery of ≥10% of spike amount.

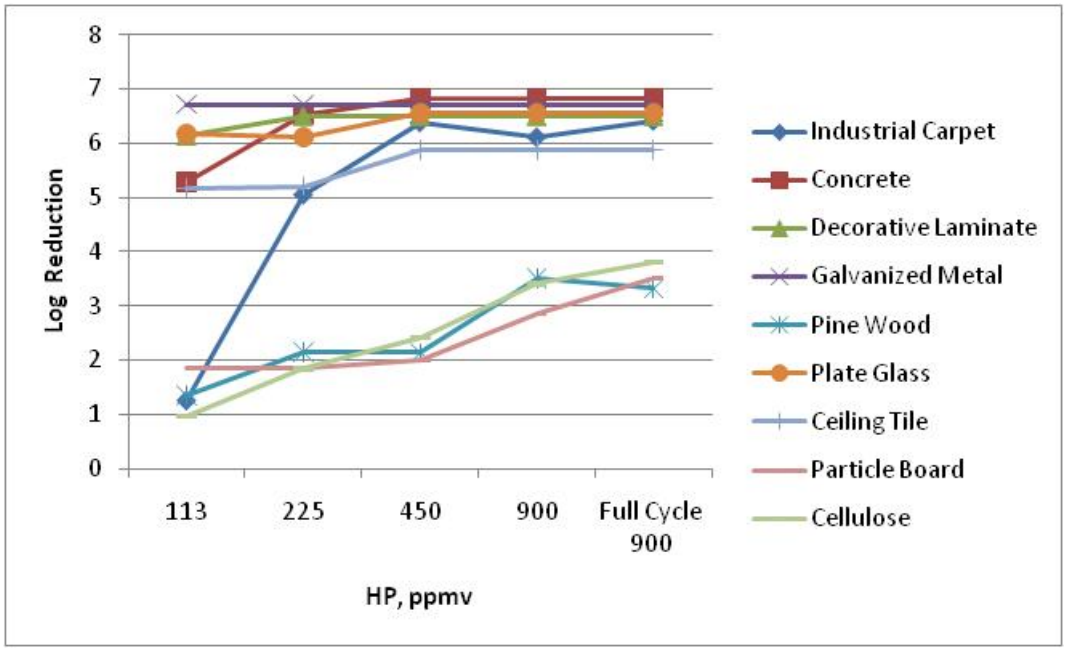


Figure 4-7. Mean *B. anthracis* log reduction from various materials vs. HP CT (225 ppmv concentration) at 22 °C.

4.3.2 Summary of Findings from the HP Investigation

- HP fumigation was efficacious for the decontamination of *B. anthracis* Ames spores from all of a broad range of indoor building materials tested.
- A CT of 2000 ppmv-hr resulted in a ≥5.7 log reduction with no viable *B. anthracis* Ames spores recovered from all building materials after a 4 hr cycle at 500 ppmv. (Higher or lower log reduction with no viable spores recovered results mathematically from the differential recovery efficiencies for control coupons among material types.) A CT of 2000 ppmv-hr is much higher than the associated HP label condition. Use of HP at 250 ppmv for 1.5 hr (a CT of 375 ppmv-hr) is the treatment specified on the STERIS Vaprox® HP sterilant package insert for sporicidal efficacy (sterilization) for pre-cleaned enclosures. The registration is for the liquid only, not the vapor.
- A CT of 500 ppmv-hr resulted in a ≥6.9 log reduction with no viable *B. anthracis* Ames spores recovered from all nonporous building materials after a 1 hr contact time at 500 ppmv (slightly higher than the associated HP label condition of 375 ppmv-hr).

5.0 Liquid Decontamination Technologies Test Results

5.1 pH-Amended Bleach

5.1.1 Qualitative Results for pH-Amended Bleach Decontamination of Bacillus Species

The qualitative cycle fraction test results are shown in Table 5-1. Three test coupons, in contact with the pH-amended bleach, and three control coupons, in contact with PBS, were included at each time point.

No viable *B. anthracis* Ames spores were observed on any material in the qualitative testing after contact with pH-amended bleach for 10 min or longer at 22 °C. Viable *B. anthracis* Ames spores were observed after contact with PBS at all time points from 10 to 60 min, except for painted concrete.

Painted concrete positive controls exhibited no growth in TSB at Day 7. An aliquot of each negative TSB painted concrete control on Day 7 was plated onto tryptic soy agar plates. All of the painted concrete positive control samples at all contact times exhibited growth of *B. anthracis* Ames when plated onto TSA; colonies were observed with morphologies consistent with the morphology of *B. anthracis* Ames colonies. No growth in TSA was observed for blank controls. Painted concrete coupons exhibited a biostatic effect in TSB as demonstrated by the growth after subsequent plating of an aliquot from the TSB that showed that viable *B. anthracis* Ames spores were present in the TSB of all positive controls.

Table 5-1. Results from Qualitative Evaluation of pH-Amended Bleach Decontamination of *B. anthracis* Ames

Material	Day 7							
	Decontaminated, Contact Time				Positive Control, Contact Time (PBS)			
	10 min	20 min	40 min	60 min	10 min	20 min	40 min	60 min
Glass	-	-	-	-	+	+	+	+
Painted Concrete	-	-	-	-	- (+*)	- (+*)	- (+*)	- (+*)
Galvanized Metal	-	-	-	-	+	+	+	+
Decorative Laminate	-	-	-	-	+	+	+	+

"-" indicates that all three coupons at the specified time and treatment exhibited no growth in TSB.
"+" indicates that all three coupons at the specified time and treatment exhibited growth in TSB.
"(+*)" indicates that when an aliquot of the negative TSB from painted concrete controls was plated onto tryptic soy agar plates on Day 7, all samples at the specified time and treatment exhibited growth of *B. anthracis* on the TSA plates.

5.1.2 *Quantitative Results for pH-amended Bleach Decontamination of Bacillus Species*

The efficacy of pH-amended bleach solutions (6,300 – 6,600 ppm total chlorine at pH 6.51 – 6.93) against three strains of *B. anthracis* spores (virulent Ames, avirulent NNR1Δ1, and Vollum) and *B. subtilis* was evaluated using the quantitative method. The spore recovery results for individual types of *Bacillus* spores exposed to pH-amended bleach or PBS for specified time periods are shown in Tables 5-2 through 5-5 and Figure 5-1. In addition, the extracted coupons were placed individually into TSB and incubated for seven days to look for cloudiness that would indicate the presence of residual viable spores on the test coupon. In all cases where no spores were observed using the quantitative methodology, no residual viable spores were detected using the qualitative method. A summary of efficacy of pH-amended bleach against the various types of spores, reported as log reduction, is shown in Table 5-6.

Efficacy was shown to be dependent on the type of material onto which the spores are inoculated. For all spore types tested, viable spores were present on carpet and particle board after exposure to pH-amended bleach for up to 10 min. Viable *B. subtilis* spores, but not *B. anthracis* Ames spores, were recovered from carpet and particle board after a 30-min exposure to pH-amended bleach. For all spore types tested, no viable spores were present on glass, galvanized metal, or decorative laminate after exposure to pH-amended bleach for 5 min or longer.

While strong similarities in efficacy were observed across spore types, differences were also observed as indicated above. For example, viable *B. anthracis*, Vollum and *B. subtilis* spores were present on painted concrete after exposure to pH-amended bleach for five min; the other two strains of *B. anthracis*, Ames and NNR1Δ1, did not have viable spores present after exposure to pH-amended bleach for 5 min. As another example of observed differences, for coupons of all materials tested, there were no viable spores recovered after 30-min exposure of *B. anthracis* Ames spores to pH-amended bleach. In contrast, viable *B. subtilis* spores were recovered from both carpet and particle board after 30-min exposure to pH-amended bleach.

Table 5-2. *B. anthracis* Ames Spores CFU after Various pH-Amended Bleach Contact Times

Material	CFU Applied to Coupons	Mean CFU (SD) Recovered from Positive Control Coupons at Specified Contact Time		
		5 min	10 min	30 min
Glass	1.07×10^7	7.65×10^6 (1.17×10^6)	5.03×10^6 (2.22×10^5)	8.43×10^6 (8.05×10^5)
Painted Concrete	1.05×10^8	1.27×10^7 (1.29×10^6)	1.41×10^7 (1.08×10^6)	9.59×10^6 (6.96×10^5)
Industrial Carpet	9.97×10^7	5.13×10^7 (2.43×10^6)	4.32×10^7 (2.40×10^6)	3.57×10^7 (2.43×10^6)
Galvanized Metal	9.97×10^7	5.07×10^7 (7.50×10^6)	4.97×10^7 (6.95×10^6)	4.24×10^7 (4.54×10^6)
Particle Board	9.90×10^7	3.79×10^7 (8.94×10^6)	3.45×10^7 (4.68×10^6)	2.55×10^7 (9.92×10^6)
Decorative Laminate	1.08×10^8	1.27×10^7 (3.12×10^6)	1.71×10^7 (1.49×10^6)	2.71×10^7 (4.90×10^6)

Material	CFU Applied to Coupons	Mean CFU (SD)* Recovered from Test Coupons at Specified Contact Time		
		5 min	10 min	30 min
Glass	1.07×10^7	0	0	0
Painted Concrete	1.05×10^8	0	0	0
Industrial Carpet	9.97×10^7	6.56×10^4 (2.39×10^4)	2.22×10^4 (2.89×10^4)	0
Galvanized Metal	9.97×10^7	0	0	0
Particle Board	9.90×10^7	6.34×10^2 (7.21×10^2)	5.92×10^2 (1.07×10^3)	0
Decorative Laminate	1.08×10^8	0	0	0

*SD not calculated when no spores are recovered from any test coupon.

Table 5-3. *B. anthracis* Vollum Spores CFU after Various pH-Amended Bleach Contact Times

Material	CFU Applied to Coupons	Mean CFU/Coupon (SD) at 5-min Contact Time	
		Positive Control Coupons	Test Coupons
Glass	1.09×10^7	6.12×10^6 (4.88×10^5)	0
Painted Concrete	1.10×10^8	5.56×10^6 (5.02×10^5)	5.88×10^2 (3.41×10^2)
Industrial Carpet	1.10×10^8	3.45×10^7 (1.81×10^6)	4.24×10^3 (6.25×10^2)
Galvanized Metal	1.09×10^8	1.95×10^7 (7.64×10^6)	0
Particle Board	1.10×10^8	1.51×10^7 (9.14×10^5)	1.04×10^3 (5.21×10^2)
Decorative Laminate	1.09×10^8	3.07×10^7 (2.36×10^6)	0

*SD not calculated when no spores are recovered from any test coupon

Table 5-4. *B. anthracis* NNR1Δ1 Spores CFU after pH-Amended Bleach Contact Times

Material	CFU Applied to Coupons	Mean CFU/Coupon (SD)* at 5-min Contact Time	
		Positive Control Coupons	Test Coupons
Glass	4.17×10^6	7.89×10^5 (1.63×10^5)	0
Painted Concrete	3.50×10^7	3.97×10^6 (9.55×10^5)	0
Industrial Carpet	2.93×10^7	8.58×10^6 (1.09×10^6)	2.07×10^1 (3.10×10^1)
Galvanized Metal	4.17×10^7	7.54×10^6 (2.06×10^6)	0
Particle Board	3.50×10^7	8.17×10^6 (1.25×10^6)	8.83×10^2 (6.38×10^2)
Decorative Laminate	4.17×10^7	8.07×10^6 (1.96×10^6)	0
Material	**CFU Applied to Coupons**	**Mean CFU/Coupon (SD)* at 10-min Contact Time**	
		Positive Control Coupons	**Test Coupons**
Particle Board	2.93×10^7	8.85×10^6 (1.31×10^6)	3.49×10^1 (7.80×10^1)

*SD not calculated when no spores are recovered from any test coupon.

Table 5-5. *B. subtilis* CFU after Various pH-Amended Bleach Contact Times

Material	CFU Applied to Coupons	Mean CFU (SD)* Recovered from Positive Control Coupons at Specified Contact Time		
		5 min	10 min	30 min
Glass	1.10×10^7	4.00×10^6 (9.16×10^5)	2.92×10^6 (2.51×10^5)	5.07×10^5 (6.01×10^4)
Painted Concrete	1.08×10^8	3.41×10^7 (1.89×10^7)	3.99×10^7 (6.75×10^6)	3.03×10^7 (2.56×10^6)
Industrial Carpet	1.06×10^8	4.54×10^7 (6.65×10^6)	5.27×10^7 (8.34×10^6)	3.77×10^7 (3.37×10^6)
Galvanized Metal	1.06×10^8	4.11×10^7 (1.03×10^7)	3.73×10^7 (7.19×10^6)	3.40×10^7 (9.56×10^6)
Particle Board	1.06×10^8	3.22×10^6 (1.32×10^6)	3.43×10^6 (1.87×10^6)	4.35×10^7 (1.99×10^6)
Decorative Laminate	1.00×10^8	6.41×10^7 (6.60×10^6)	5.74×10^7 (5.30×10^6)	4.35×10^7 (1.18×10^7)
Material	**CFU Applied to Coupons**	**Mean CFU (SD)* Recovered from Test Coupons at Specified Contact Time**		
		5 min	**10 min**	**30 min**
Glass	1.10×10^7	0	0	0
Painted Concrete	1.08×10^8	1.39×10^1 (3.10×10^1)	0	0
Industrial Carpet	1.06×10^8	6.45×10^4 (1.48×10^4)	5.43×10^3 (1.31×10^3)	2.90×10^3 (1.39×10^3)
Galvanized Metal	1.06×10^8	0	0	0
Particle Board	1.06×10^8	3.83×10^3 (1.17×10^3)	1.33×10^3 (9.11×10^2)	9.04×10^1 (3.96×10^1)
Decorative Laminate	1.00×10^8	0	0	0

*SD not calculated when no spores are recovered from any test coupon

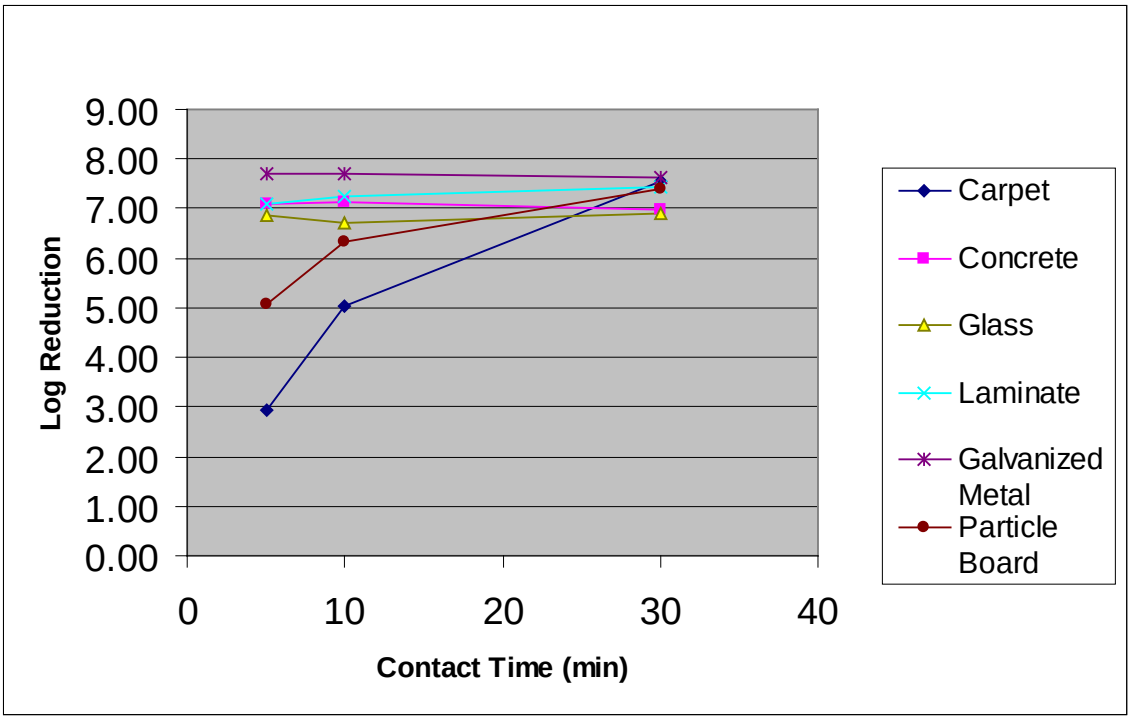


Figure 5-1. Decontamination efficacy of pH-amended bleach against *B. anthracis* Ames spores at 22 °C.

A further comparison of the efficacy of pH-amended bleach at a 5-min contact time was conducted for the decontamination of *B. anthracis* Ames spores and three surrogates (*B. anthracis* Vollum, *B. anthracis* NNR1Δ1, and *B. subtilis* spores). The efficacy was determined for each of the six indoor building materials tested (both porous and nonporous). As shown in Figure 5-2, after a 5-min contact time with pH-amended bleach the results were similar for the biological agent (*B. anthracis* Ames spores) and the three surrogates. For the biological agent and the three surrogates, no viable spores were recovered from any of the hard, nonporous building materials after a 5-min exposure to pH-amended bleach. Shown in Table 5-7, greater than a 6 log reduction in viable *B. anthracis* spores (Ames, Vollum, and NNR1Δ1) and *B. subtilis* spores was observed from all hard, nonporous building materials (glass, galvanized metal, and decorative laminate), except *B. anthracis* NNR1Δ1 spores on glass. While no viable *B. anthracis* NNR1Δ1 spores were recovered after a 5-min contact time, only a 5.89 log reduction was observed (maximum possible) because a lower CFU inoculation of NNR1Δ1 spores (4.2×10^6) was applied to the coupon than the 1.1×10^7 CFU of spores applied for Ames and Vollum.

Likewise, for carpet and particle board, a 2.86 or greater log reduction in viable spores was observed for the biological agent and three surrogates after a 5-min contact time. Viable spores of the biological agent and three surrogates were recovered from one or more of the replicate coupons.

A 30-min contact time with pH-amended bleach resulted in a >6 log reduction in viable *B. anthracis* Ames spores (no CFU observed) from all building materials tested. *B. subtilis* spores on carpet and particle board after a 30-min contact time with pH-amended bleach only exhibited a 4.2 to 4.7 log reduction; viable spores were still recovered. *B. subtilis* appears to be a conservative surrogate for *B. anthracis* Ames when pH-amended bleach is used.

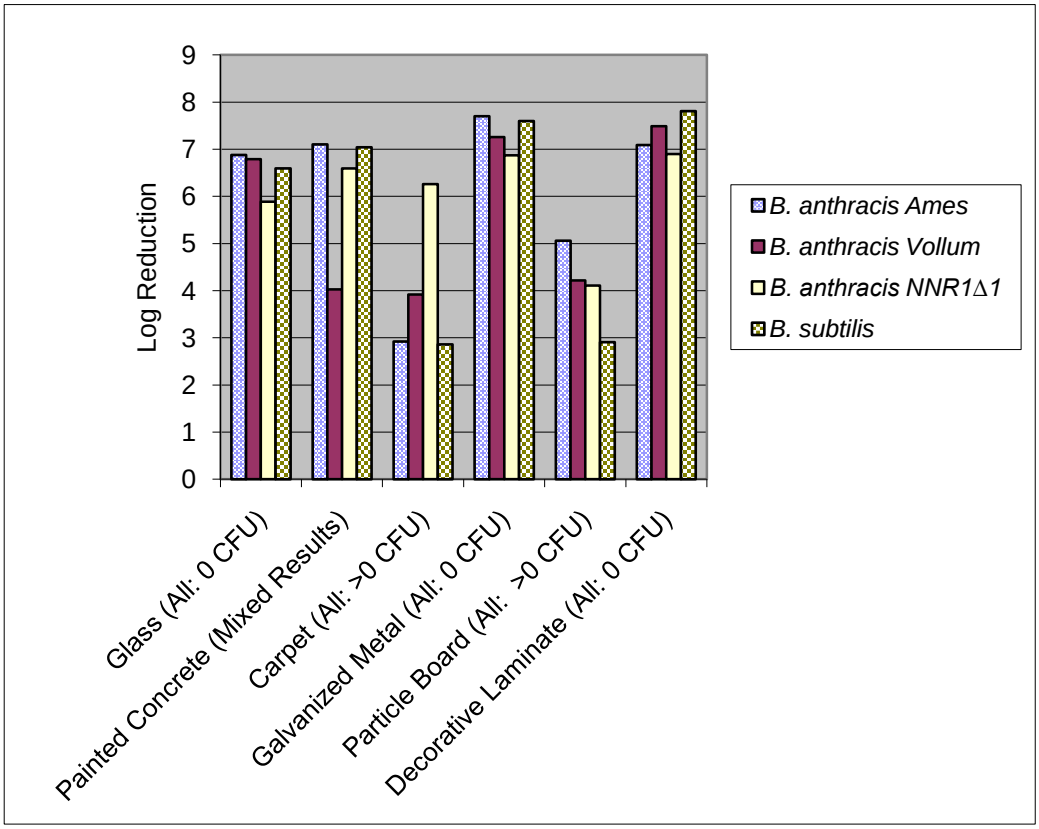


Figure 5-2. Decontamination efficacy of pH-amended bleach against *B. anthracis* spores and surrogates with 5-min contact time.

Table 5-6. Summary of pH-Amended Bleach Decontamination of Various *Bacillus* Species and Strains

Material	Spore	Mean Log Reduction (95% Confidence Interval), and p- Value After Decontamination for Specified Contact Time		
		5 min	10 min	30 min
Glass	*B. anthracis* Ames	**>6.88 p=0.014**	**>6.70 p=0.014**	**>6.92 p=0.014**
	B. anthracis Vollum	**>6.79 p=0.014**	**NA**	
	B. anthracis NNR1Δ1	**>5.89 p=0.014**	**NA**	
	B. subtilis	**>6.59 p=0.014**	**>6.46 p=0.014**	**>5.70 p=0.014**
Painted Concrete	*B. anthracis* Ames	**>7.10 p=0.014**	**>7.15 p=0.014**	**>6.98 p=0.014**
	B. anthracis Vollum	**4.03 (3.78, 4.28) p<0.0001**	**NA**	
	B. anthracis NNR1Δ1	**>6.59 p=0.014**	**NA**	
	B. subtilis	**>7.04 p=0.014**	**>7.60 p=0.014**	**>7.48 p=0.014**
Carpet	*B. anthracis* Ames	**2.92 (2.73, 3.11) p<0.0001**	**>5.03 p=0.014**	**>7.55 p=0.014**
	B. anthracis Vollum	**3.92 (3.85, 4.00) p<0.0001**	**NA**	
	B. anthracis NNR1Δ1	**6.26 p=0.014**	**NA**	
	B. subtilis	**2.86 (2.73, 2.98) p<0.0001**	**3.99 (3.85, 4.14) p<0.0001**	**4.16 (3.91, 4.41) p<0.0001**
Galvanized Metal	*B. anthracis* Ames	**>7.70 p=0.014**	**>7.69 p=0.014**	**>7.63 p=0.014**
	B. anthracis Vollum	**>7.26 p=0.014**	**NA**	
	B. anthracis NNR1Δ1	**>6.87 p=0.014**	**NA**	
	B. subtilis	**>7.60 p=0.014**	**>7.57 p=0.014**	**>7.52 p=0.014**
Particle Board	*B. anthracis* Ames	**5.06 (4.38, 5.73) p<0.0001**	**>6.32 p=0.014**	**>7.38 p=0.014**
	B. anthracis Vollum	**4.22 (3.94, 4.50) p<0.0001**	**NA**	
	B. anthracis NNR1Δ1	**4.11 (3.64, 4.58) p<0.0001**	**NA**	
	B. subtilis	**2.91 (2.64, 3.17) p<0.0001**	**3.41 (3.00, 3.82) p<0.0001**	**4.67 (4.31, 5.04) p<0.0001**
Decorative Laminate	*B. anthracis* Ames	**>7.09 p=0.014**	**>7.23 p=0.014**	**>7.43 p=0.014**
	B. anthracis Vollum	**>7.49 p=0.014**	**NA**	
	B. anthracis NNR1Δ1	**>6.90 p=0.014**	**NA**	
	B. subtilis	**>7.81 p=0.014**	**>7.76 p=0.014**	**>7.63 p=0.014**

NA = not applicable

5.1.3 *Quantitative Results for pH-Amended Bleach Efficacy Decontamination of Vaccinia Virus*

The efficacy of pH-amended bleach (6,000 – 6,700 ppm total chlorine at pH 6.51 – 6.55) against vaccinia virus was evaluated using the quantitative method by counting PFU after a given treatment. The recovery of viable vaccinia virus exposed to pH-amended bleach (decontamination) or PBS (positive control) for a 5-min contact time is shown in Table 5-7. The efficacy of the 5-min pH-amended bleach treatment, shown as log reduction, is included in Table 5-7. Using the plaque assay, no viable vaccinia virus was observed on coupons of any material type after a 5-min contact time with pH-amended bleach.

Table 5-7. Vaccinia PFU after 5 Min pH-amended Bleach Contact Time

Material	PFU Applied to Coupons	Mean PFU/ Positive Control Coupon (SD) (0 Min)	Mean PFU/ Positive Control Coupon (SD), 5-min Contact Time with PBS	Mean PFU/Test Coupon (SD) at 5-min Contact Time[#]	Differential Log Reduction Compared to Controls, Statistical p-value*[H]
Glass	7.37×10^6	1.61×10^5 (7.86×10^4)	6.32×10^4 (2.84×10^4)	0	**> 4.76 p=0.0135**
Painted Concrete	7.37×10^6	1.23×10^6 (7.86×10^5)	1.89×10^5 (2.81×10^4)	0	**> 5.27 p=0.0135**
Industrial Carpet	7.37×10^6	1.15×10^5 (1.63×10^5)	6.61×10^4 (1.58×10^4)	0	**> 4.81 p=0.0135**
Galvanized Metal	7.37×10^6	9.45×10^5 (1.43×10^5)	3.00×10^2 (6.16×10^2)	0	Indeterminate[‡] p=0.8186
Particle Board	7.37×10^6	1.53×10^3 (1.04×10^3)	3.59×10^2 (3.86×10^2)	0	**> 2.34 p=0.0135**
Decorative Laminate	7.37×10^6	4.88×10^5 (1.20×10^5)	2.15×10^4 (1.46×10^4)	0	**> 4.20 p=0.0135**

*Log reduction values are calculated by taking the mean of the $\log_{10}$ recovered values of the positive control coupons at 5 min contact time with PBS and subtracting the mean of the $\log_{10}$ recovered values of the 5 min treated coupons with 1 PFU substituted for any observed 0 values. In cases where only decontaminated coupons have observed zero values, this procedure makes the log reduction a minimum value as indicated by the "> x" reported value.

[H]The statistical result comes from comparing the five recovered values from the positive controls at 5 min contact time to the five recovered values from the treated coupons (all zeros) using a nonparametric Kolmogorov-Smirnov test. Values less than 0.05 indicates the reduction due to the pH-amended bleach treatment for 5 min was superior to the reduction of the controls for 5 min.

[‡]With zero values in both the positive control group and the decontamination group, the estimated log reduction is indeterminate.

[#]SD not calculated when no viable viruses are recovered from any test coupon.

5.1.4 *Summary of Findings from the pH-Amended Bleach Investigation*

- Contact with pH-amended bleach was efficacious (significant log reduction was observed) for the decontamination of *B. anthracis* Ames spores from the six indoor building materials tested (both porous and nonporous); efficacy increased with increasing contact time.
- A contact time of 5 min with pH-amended bleach at 22 °C resulted in a >6 log reduction in viable *B. anthracis* spores (Ames and Vollum) and *B. subtilis* spores from all hard, nonporous building materials; no viable *B. anthracis* NNR1Δ1 spores were recovered after 5 min, but a 5.89 log reduction was observed. The relatively lower log reduction was due to a lower CFU inoculation of NNR1Δ1 spores (4.2×10^6) being applied to the coupon rather than 1.1×10^7 CFU applied for Ames and Vollum. Previous EPA testing with spray-applied pH-amended bleach (60 min contact time) was also found to be effective against *B. anthracis* and *B. subtilis* spores on painted cinder block (log reductions >7.0).[32]
- A contact time of 30 min with pH-amended bleach at 22 °C resulted in a >6 log reduction in viable *B. anthracis* Ames spores (no CFU observed) from all building materials tested.
- In contrast to *B. anthracis* Ames spores where no CFU were observed on any material after a contact time of 30 min or less at 22 °C, only a 4.2 to 4.7 log reduction in *B. subtilis* spores on carpet and particle board was observed after 30 min contact with pH-amended bleach at 22 °C.
- A contact time of 5 min with pH-amended bleach at 22 °C resulted in no PFU of vaccinia virus being recovered from the six indoor building materials tested (both porous and nonporous).

5.2 Liquid ClO_2

5.2.1 *Results for Liquid ClO_2 Decontamination of Bacillus Species*

The efficacy of liquid ClO_2 was evaluated when applied to *B. anthracis* Ames spores and *B. subtilis* spores on coupons of four building materials. The four building materials (glass, industrial carpet, particle board, and decorative laminate) were selected from, and identical to, coupons used in the pH-amended bleach testing described in Section 5.1. The recovery results for individual types of *Bacillus* spores exposed to liquid ClO_2 or PBS for specified time periods at 20 °C are shown in Tables 5-8 and 5-9. In addition, the extracted coupons were placed individually into TSB and incubated for seven days to look for cloudiness that would indicate the presence of residual viable spores on the test coupon. In all cases where no spores were observed using the quantitative methodology, no residual viable spores were detected using the qualitative method. The efficacy of liquid ClO_2 against *B. anthracis* Ames spores and *B. subtilis* spores, reported as log reduction, is shown in Table 5-10.

Efficacy was shown to be dependent on the type of material onto which the spores were applied. For *B. anthracis* and *B. subtilis* spores, viable spores were recovered from particle board after exposure to liquid ClO_2 for 120 min at 22 °C. Likewise, viable *B. anthracis* Ames and *B. subtilis* spores were recovered from carpet after exposure to liquid ClO_2 for 30 min at 22 °C. Viable *B. subtilis* spores, but not *B. anthracis* Ames, were recovered from carpet after a 120-min exposure to liquid ClO_2 at 22 °C.

A complete kill of spores on glass, industrial carpet, and decorative laminate, i.e., no viable spores recovered (quantitative analysis) or detected (qualitative analysis), occurred at a lower contact time with liquid ClO_2 at 22 °C for *B. anthracis* Ames spores than for *B. subtilis* spores.

Exterm ClO_2 solution provided >6 log reduction in recoverable viable *B. anthracis* Ames spores, shown in Figure 5-3, dependent on the building material and the contact time at 22 °C. No viable *B. anthracis* Ames spores were recovered from industrial carpet, decorative laminate, or glass after 120 min exposure to the ClO_2 solution at 22 °C; viable *B. anthracis* Ames spores were recovered from particle board after 120 min exposure to ClO_2 solution at 22 °C. Shown in Figure 5-4, Exterm ClO_2 solution provided >6 log reduction in *B. subtilis* spores (no viable spores recovered) from glass and decorative laminate (hard, nonporous surfaces) after 120 min exposure to ClO_2 solution at 22 °C. Viable *B. subtilis* spores were recovered from both industrial carpet and particle board (porous surfaces) after 120 min exposure to ClO_2 solution at 22 °C. In general, *B. subtilis* spores appear to be able to survive a longer exposure to ClO_2 solution at 22 °C than *B. anthracis* Ames spores.

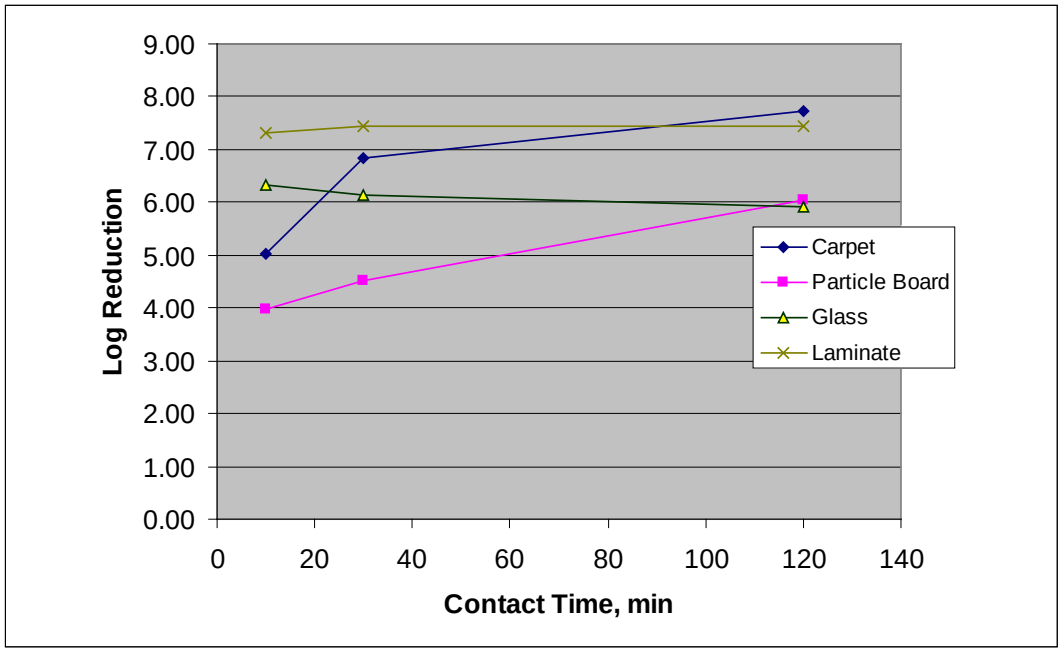


Figure 5-3. Decontamination efficacy of Exterm ClO_2 solution against *B. anthracis* Ames spores at 22 °C.

Table 5-8. *B. anthracis* Ames spores CFU after Various Liquid ClO_2 Contact Times

Material	*B. anthracis* (Ames) CFU Applied to Coupons	Mean CFU (SD) Recovered from Positive Control Coupons at Specified Contact Time with PBS		
		10 min	30 min	120 min
Glass	1.09×10^7	2.08×10^6 (1.37×10^6)	1.39×10^6 (7.50×10^5)	8.57×10^5 1.70×10^5)
Industrial Carpet	1.09×10^8	6.11×10^7 (1.00×10^7)	4.50×10^7 (1.26×10^7)	5.45×10^7 (1.73×10^7)
Particle Board	1.11×10^8	3.38×10^7 (6.14×10^6)	3.93×10^7 (6.35×10^6)	3.66×10^7 (1.03×10^7)
Decorative Laminate	1.03×10^8	2.07×10^7 (8.04×10^6)	2.79×10^7 (1.30×10^7)	2.69×10^7 (8.96×10^6)
Material	***B. anthracis* (Ames) CFU Applied to Coupons**	**Mean CFU (SD)* Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**30 min**	**120 min**
Glass	1.09×10^7	0	0	0
Industrial Carpet	1.09×10^8	6.11×10^2 (2.93×10^2)	4.19×10^1 (5.92×10^1)	0
Particle Board	1.11×10^8	3.59×10^3 (6.16×10^2)	1.28×10^3 (5.32×10^2)	6.95×10^1 (5.50×10^1)
Decorative Laminate	1.03×10^8	6.65×10^0 (1.49×10^1)	0	0

*SD not calculated when no viable virus are recovered from any test coupon.

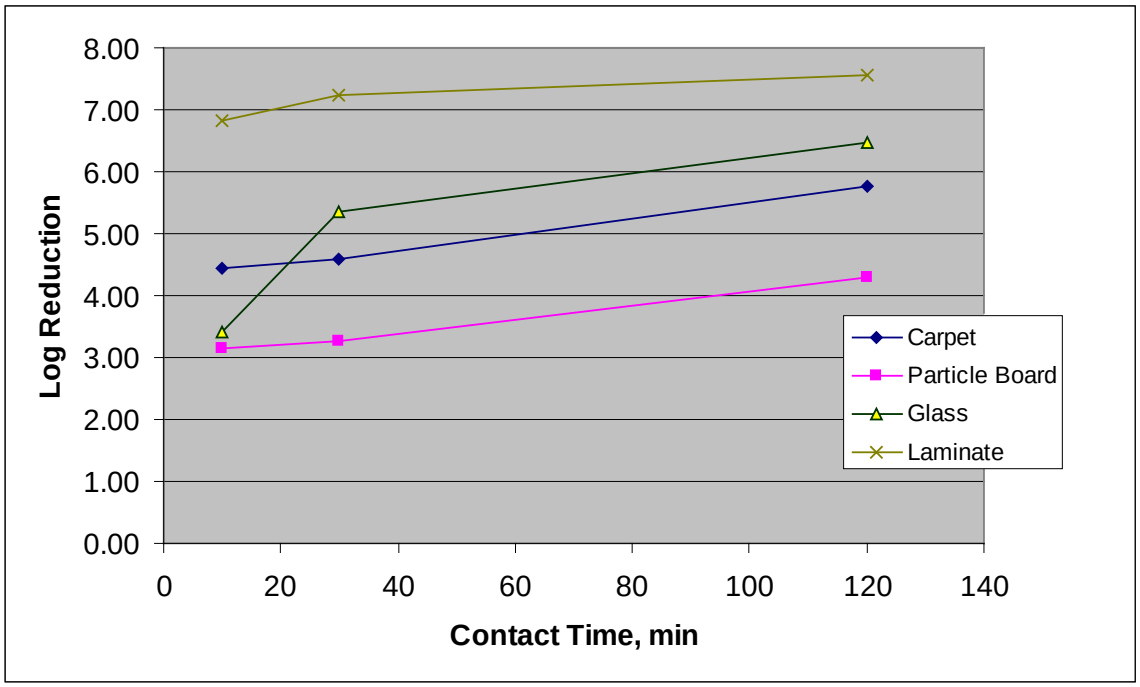


Figure 5-4. Decontamination efficacy of Exterm ClO_2 solution against *B. subtilis* spores.

Table 5-9. *B. subtilis* CFU after Various Contact Times with Liquid ClO_2

Material	*B. subtilis* CFU Applied to Coupons	Mean CFU (SD) Recovered from Positive Control Coupons at Specified Contact Time with PBS		
		10 min	30 min	120 min
Glass	1.01×10^7	3.67×10^6 (7.55×10^5)	4.00×10^6 (1.14×10^6)	2.92×10^6 (1.49×10^5)
Industrial Carpet	1.07×10^8	2.40×10^7 (6.48×10^6)	2.51×10^7 (5.14×10^6)	3.48×10^7 (6.85×10^6)
Particle Board	1.00×10^8	4.13×10^6 (9.57×10^5)	3.47×10^6 (3.95×10^5)	4.59×10^6 (1.03×10^6)
Decorative Laminate	1.02×10^8	4.27×10^7 (4.33×10^6)	3.90×10^7 (4.02×10^6)	3.57×10^7 (6.58×10^6)
Material	***B. subtilis* CFU Applied to Coupons**	**Mean CFU (SD)* Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**30 min**	**120 min**
Glass	1.01×10^7	1.44×10^3 (2.03×10^2)	1.01×10^3 (3.99×10^2)	0
Industrial Carpet	1.07×10^8	1.39×10^3 (1.23×10^3)	1.48×10^3 (1.80×10^3)	2.09×10^2 (2.38×10^2)
Particle Board	1.00×10^8	2.96×10^3 (5.03×10^2)	1.91×10^3 (3.91×10^2)	2.57×10^2 (1.12×10^2)
Decorative Laminate	1.02×10^8	6.71×10^1 (1.32×10^2)	1.35×10^1 (3.02×10^1)	0

*SD not calculated when no viable virus are recovered from any test coupon.

Table 5-10. Log Reduction in *Bacillus* Species after Various Contact Times with Liquid ClO_2

Material	Organism	Mean Log Reduction* (95% Confidence Interval[H]), p-value[ŧ] After Decontamination for Specified Contact Time		
		10 min	30 min	120 min
Decorative Laminate	*B. anthracis* Ames	**> 6.99 p=0.0135**	**> 7.38 p=0.0135**	**> 7.40 p=0.0135**
	B. subtilis	**> 6.83 p=0.0135**	**> 7.22 p=0.0135**	**> 7.55 p=0.0135**
Industrial Carpet	*B. anthracis* Ames	**5.03 (4.81, 5.26) p < 0.0001**	**> 6.83 p=0.0135**	**> 7.72 p=0.0135**
	B. *subtilis*	**4.44 (3.87, 5.01) p < 0.0001**	**4.58 (3.89, 5.27) p < 0.0001**	**> 5.76 p=0.0135**
Glass	*B. anthracis* Ames	**> 6.24 p=0.0135**	**> 6.10 p=0.0135**	**> 5.93 p=0.0135**
	B. subtilis	**3.40 (3.29, 3.51) p < 0.0001**	**3.62 (3.36, 3.89) p < 0.0001**	**> 6.47 p=0.0135**
Particle Board	*B. anthracis* Ames	**3.97 (3.86, 4.09) p < 0.0001**	**4.51 (4.30, 4.72) p < 0.0001**	**> 6.04 p=0.0135**
	B. subtilis	**3.14 (3.01, 3.27) p < 0.0001**	**3.27 (3.16, 3.37) p < 0.0001**	**4.27 (4.05, 4.50) p < 0.0001**

*Reduction calculated as $\log_{10}$ of the arithmetic mean of five control coupons minus the $\log_{10}$ of the arithmetic mean of the five decontaminated coupons. If decontaminated coupons showed zero organisms, the observed values are replaced with one.

[H]Applies only for conditions where control and decontamination coupons had nonzero results.

ŧFor cases with measurable control and decontamination results, p-value is a result of t-test comparison of control to decontamination with values less than or equal to 0.05 representing statistically significant decontamination effect. For cases with complete kill observed on at least one coupon, the p-value is a result of the nonparametric Kolmogorov-Smirnov test of whether the residual organisms after decontamination are different from the controls. Values less than or equal to 0.05, with positive mean log reductions, indicate that a positive and statistically significant reduction occurred as a result of the decontamination treatment.

5.2.2 Results for Liquid ClO_2 Decontamination of Vaccinia Virus

The efficacy of liquid ClO_2 against vaccinia virus was evaluated using the quantitative method by counting PFU after a given treatment. The recovery of viable vaccinia virus exposed to liquid ClO_2 (decontamination) or PBS (positive control) for a 10-min contact time is shown in Table 5-11. The efficacy of the 10-min liquid ClO_2 treatment at 22 °C, shown as log reduction, is included in Table 5-11. Using the plaque assay, no viable vaccinia virus was observed on any coupon of any material type after a 10-min contact time with liquid ClO_2 at 22 °C

Table 5-11. Vaccinia PFU after 10-Min Contact with Liquid ClO_2

Material	Vaccinia PFU Applied to Coupons	Mean (SD) PFU Recovered from Positive Control Coupons, at 0 min (No Contact Time)	Mean (SD) PFU Recovered from Positive Control Coupons, at 10 min Contact Time with PBS	Mean PFU‡ Recovered from Coupons After Decontamination at 10 min Contact Time	ClO_2 Differential Log Reduction Compared to Controls, Statistical p-Value*[H]
Glass	1.14×10^6	3.91×10^5 (1.36×10^5)	2.65×10^5 (1.31×10^5)	0	**> 5.36 p=0.0135**
Industrial Carpet	1.14×10^7	2.37×10^5 (1.84×10^5)	5.61×10^4 (3.31×10^4)	0	**> 4.38 p=0.0135**
Particle Board	1.05×10^7	1.41×10^3 (1.01×10^3)	9.52×10^2 (1.58×10^3)	0	**> 2.54 p=0.0135**
Decorative Laminate	1.14×10^7	8.41×10^5 (1.80×10^5)	6.93×10^4 (1.24×10^4)	0	**> 4.84 p=0.0135**

*Log reduction values are calculated by taking the mean of the $\log_{10}$ recovered values of the positive control coupons at 10 min contact time and subtracting the mean of the $\log_{10}$ recovered values of the 10 min treated coupons with 1 PFU substituted for the observed 0 values. This procedure makes the log reduction a minimum value as indicated by the "> x" reported value.

[H]The statistical result comes from comparing the five recovered values from the positive controls at 10 min contact time to the five recovered values from the treated coupons (all zeros) using a nonparametric Kolmogorov-Smirnov test. Values less than 0.05 indicates the reduction due to the ClO_2 treatment for 10 min was superior to the reduction of the controls for 10 min.

‡SD not calculated when no viable viruses are recovered from any test coupon.

While approximately 10^7 PFU of vaccinia virus were applied to the test control coupons (except small glass), the number of recoverable PFU on control coupons drops by about 1 to 4 log (depending on the material) within 10 min. Therefore, even with no recoverable virus (0 PFU) from test coupons, the log reduction attributable to the treatment was moderate. For example, about 2.5 for particle board. Other unknown factors contribute to the loss of viable virus in the absence of the ClO_2 treatment.

5.2.3 Summary of Findings from the Liquid ClO_2 Investigation

- Contact with liquid ClO_2 at 22 °C was efficacious for the decontamination of *B. anthracis* Ames spores, *B. subtilis* spores, and vaccinia virus from the four indoor building materials tested (glass, industrial carpet, particle board, and decorative laminate, although complete kills of *B. anthracis* Ames spores on particle board or *B. subtilis* spores on carpet or particle board did not occur even after 120 min contact times).
- A 10-min contact time with liquid ClO_2 at 22 °C resulted in a >6 log reduction in viable *B. anthracis* Ames spores on the hard, nonporous building materials (glass and decorative laminate).
- In contrast to *B. anthracis* Ames, >6 log reduction in viable *B. subtilis* spores on decorative laminate and glass was not observed at 10-min contact time at 22 °C, but was observed after

30-min and 120-min contact times at 22 °C, respectively.

- After a 120-min contact time at 22 °C, viable *B. subtilis* spores were cultured from carpet and particle board; at 120 min carpet showed a >5.7 log reduction and particle board showed a 4.3 log reduction in viable *B. subtilis* spores.
- For these materials, *B. subtilis* appears to be a conservative surrogate for decontamination of *B. anthracis* Ames spores using liquid ClO_2.
- A 10-min contact time with liquid ClO_2 at 22 °C resulted in no culturable vaccinia virus being recovered (0 PFU) from the four indoor building materials tested (both porous and nonporous).

5.3 Spor-Klenz® Ready-to-Use HP-PA Solution

5.3.1 Qualitative Results for Spor-Klenz® HP-PA Decontamination of Bacillus Species

The qualitative cycle fractionation test results are shown in Table 5-12. Coupons were inoculated with 6.8×10^7 spores per coupon (slightly below the specified target application range of 7.5×10^7 - 1.25×10^8). Three test coupons, in contact with the Spor-Klenz® HP-PA solution were included at each time point. Viable *B. anthracis* Ames spores were observed after contact with Spor-Klenz® HP-PA for up to 30 min. Some of the replicate decorative laminate coupons were negative for growth after 20-min and 30-min contact times with Spor-Klenz® HP-PA at 20 °C. Viable *B. anthracis* Ames spores were observed on all positive control coupons after contact with PBS for 5, 10, 20, or 30 min at 20 °C.

Table 5-12. Results from Qualitative Evaluation of Spor-Klenz® HP-PA Decontamination of *B. anthracis* Ames

Material	Day 7 Decontaminated, Contact Time			
	5 min	10 min	20 min	30 min
Glass	+	+	+	+
Painted Concrete	+	+	+	+
Galvanized Metal	+	+	+	+
Decorative Laminate	+	+	+, -, -	+, +, -
Positive Control	+	+	+	+
Negative Control	-	-	-	-

"-" indicates that all three coupons at the specified time and treatment exhibited no growth in TSB.
"+" indicates that all three coupons at the specified time and treatment exhibited growth in TSB.
"+, +, -" indicates two coupons exhibited growth in TSB, one coupon did not.
"+, -, -" indicates one coupon exhibited growth in TSB, two coupon did not.

5.3.2 Quantitative Results for Spor-Klenz® HP-PA Decontamination of Bacillus Species

The efficacy of Spor-Klenz® HP-PA decontamination was evaluated when applied to *B. anthracis* Ames spores on coupons of six building materials and when applied to *B. subtilis* on glass. The six building materials (glass, painted concrete, industrial grade carpet, galvanized metal ductwork, particle board, and decorative laminate) were selected from, and to the extent feasible identical to, coupons used in the pH-amended bleach testing described in Section 5.1. The recovery results from various materials for individual types of *Bacillus* spores exposed to Spor-Klenz® HP-PA (for test coupons) or PBS (for positive control and procedural blank coupons) for specified time periods are shown in Tables 5-13 and 5-14. The efficacy of Spor-Klenz® HP-PA against *B. anthracis* Ames and *B. subtilis* spores, reported as log reduction, is shown in Table 5-15 and Figure 5-5.

Efficacy was shown to be dependent on the type of material onto which the spores were applied. For both *B. anthracis* Ames and *B. subtilis*, spores were recovered from only one of five glass coupons after 10-min contact time with Spor-Klenz® HP-PA at 20 °C; no spores were recovered from any glass coupons after a 20-30 min contact time at 20 °C. A complete kill of spores on decorative laminate, i.e., no viable spores were recovered (quantitative analysis) or detected (qualitative analysis), occurred at a 30-min contact time with Spor-Klenz® HP-PA at 20 °C for *B. anthracis* Ames spores. In contrast, less than a 2-log reduction in viable spores was observed after a 30-min contact time with Spor-Klenz® HP-PA at 20 °C for *B. anthracis* Ames spores on galvanized metal. Spor-Klenz® HP-PA efficacy was greater against *B. anthracis* Ames spores on carpet than on metal, but after a 30-min contact time with Spor-Klenz® HP-PA at 20 °C less than a 4-log reduction was observed.

Table 5-13. *B. anthracis* Ames Spores CFU after Various Contact Times with Spor-Klenz® HP-PA

Material	CFU Applied to Coupons[#]	Mean CFU (SD) Recovered from Positive Control Coupons		
Glass	1.80×10^8	7.96×10^7 (9.10×10^6)		
Painted Concrete	1.80×10^8	7.18×10^7* (3.04×10^7)		
Indistrial Carpet	1.80×10^8	9.40×10^7 (6.16×10^6)		
Galvanized Metal	2.00×10^8	1.62×10^8 (2.05×10^7)		
Particle Board	2.00×10^8	1.22×10^8 (1.48×10^7)		
Decorative Laminate	2.00×10^8	1.26×10^8 (2.30×10^7)		
Material	**CFU Applied to Coupons[‡]**	**Mean CFU (SD)[†] Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**20 min**	**30 min**
Glass	1.80×10^8	6.00×10^0 (1.34×10^1)	0	0
Painted Concrete	1.80×10^8	1.97×10^6* (3.25×10^6)	8.67×10^5 (1.86×10^6)	3.28×10^6 (6.57×10^6)
Carpet	1.80×10^8	3.08×10^5 (2.76×10^5)	1.82×10^4 (2.15×10^4)	5.20×10^2 (5.97×10^2)
Galvanized Metal	2.00×10^8	1.10×10^7 (3.45×10^6)	8.54×10^6 (3.47×10^6)	6.98×10^6 (4.52×10^6)
Particle Board	2.00×10^8	1.03×10^5 (2.90×10^4)	1.07×10^3 (1.01×10^3)	4.00×10^2 (5.87×10^2)
Decorative Laminate	2.00×10^8	1.92×10^5 (5.12×10^4)	Data not valid	0

*Only three, rather than five, control coupons were included.
[†]SD not calculated when no viable spores (measured as CFU) were recovered from any test coupon.
[‡]The number of spores inoculated onto the coupons was slightly above the specified range of 7.5×10^7 - 1.25×10^8.

Table 5-14. *B. anthracis* Ames and *B. subtilis* Spores CFU after Various Contact Times with Spor-Klenz® HP-PA

Material	*B. anthracis* (Ames) CFU Applied to Coupons	Mean CFU (SD) Recovered from Positive Control Coupons		
Glass	1.80×10^8	7.96×10^7 (9.10×10^6)		
Material	***B. anthracis* (Ames) CFU Applied to Coupons**	**Mean CFU (SD)* Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**20 min**	**30 min**
Glass	1.80×10^8	6.0×10^0 (1.34×10^1)	0	0
Material	***B. subtilis* CFU Applied to Coupons**	**Mean CFU (SD) Recovered from Positive Control Coupons**		
Glass	1.20×10^8	7.16×10^6 (3.45×10^6)		
Material	***B. subtilis* CFU Applied to Coupons**	**Mean CFU (SD)* Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**20 min**	**30 min**
Glass	1.20×10^8	6.00×10^0 (1.34×10^1)	0	0

*SD not calculated when no viable spores (measured as CFU) were recovered from any test coupon.
†The number of spores inoculated onto the coupons was slightly above the specified range of 7.5×10^7 - 1.25×10^8).

Table 5-15. Log Reduction in *Bacillus* Species after Various Contact Times with Spor-Klenz® HP-PA

Material	Spore	Mean Log Reduction* After Decontamination for Specified Contact Time		
		10 min	**20 min**	**30 min**
Glass	*B. anthracis* Ames	7.62	>7.91	>7.91
	B. subtilis	6.54	>6.83	>6.83
Painted Concrete	*B. anthracis* Ames	2.52	4.25	2.42
Carpet	*B. anthracis* Ames	2.62	4.20	6.26
Galvanized Metal	*B. anthracis* Ames	1.15	1.28	1.46
Particle Board	*B. anthracis* Ames	3.09	5.32	6.89
Decorative Laminate	*B. anthracis* Ames	2.78	Data not valid	>8.05

*Log reduction values are calculated by taking the mean of the $\log_{10}$ recovered values of the positive control coupons and subtracting the mean of the $\log_{10}$ recovered values of the treated coupons at a given contact time with 1 CFU substituted for the observed 0 values. This procedure makes the log reduction a minimum value as indicated by the "> x" reported value.

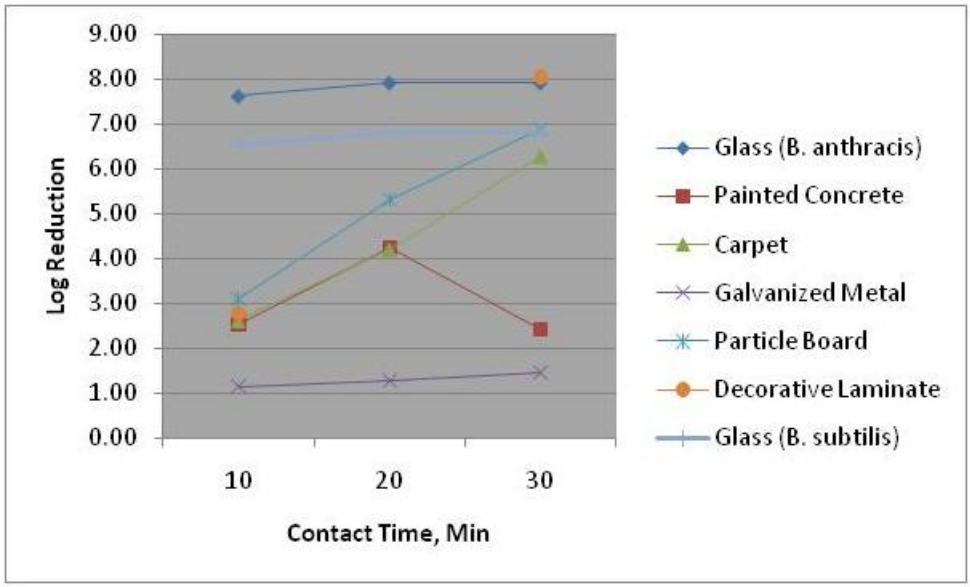


Figure 5-5. Decontamination efficacy of Spor-Klenz® HP-PA solution against *B. anthracis* Ames on various materials and *B. subtilis* spores on glass.

5.3.3 Summary of Findings from the Spor-Klenz® HP-PA Investigation

- Contact with Spor-Klenz® HP-PA exhibits variable efficacy for the decontamination of *B. anthracis* Ames spores from the six indoor building materials tested (both porous and nonporous).
- A 30-min contact time with Spor-Klenz® HP-PA at 20 °C resulted in a >6 log reduction in viable *B. anthracis* Ames spores from all materials tested except painted concrete and galvanized metal.
- *B subtilis* spores on glass showed the same pattern of decontamination efficacy as *B. anthracis* Ames spores on glass and appears to be a good surrogate for decontamination of *B anthracis* Ames using Spor-Klenz® HP-PA.

5.4 Oxonia Active® Solution

5.4.1 Quantitative Results for Oxonia Active® Decontamination of Bacillus Species

The efficacy of Oxonia Active® decontamination was evaluated when applied to *B. anthracis* Ames spores on coupons of six building materials and when applied to *B. subtilis* on glass. The six building materials (glass, painted concrete block, industrial grade carpet, galvanized metal ductwork, particle board, and decorative laminate) were selected from, and to the extent feasible identical to, coupons used in the pH-amended bleach testing described in Section 5.1. The recovery results for individual types of *Bacillus* spores exposed to Oxonia Active® (for test coupons) or PBS (for positive controls) for specified time periods are shown in Tables 5-16 and 5-17. The efficacy of Oxonia Active® against *B. anthracis* Ames and *B. subtilis* spores, reported as

log reduction, is shown in Table 5-18 and Figure 5-6.
Efficacy was shown to be dependent on the type of material onto which the spores were applied. No *B. anthracis* Ames spores were recovered from any galvanized metal or particle board coupons after a 30- or 60-min contact time at 20 °C. Viable *B. anthracis* spores were recovered from one or more coupons of glass, painted concrete block, industrial grade carpet, and decorative laminate after a 60-min contact time with Oxonia Active® at 20 °C.

5.4.2 Summary of Findings from the Oxonia Active® Investigation

- Contact with Oxonia Active® exhibits variable efficacy for the decontamination of *B. anthracis* Ames spores from the six indoor building materials tested (both porous and nonporous).
- A 30-min contact time with Oxonia Active® at 20 °C resulted in a >6 log reduction in viable *B. anthracis* Ames spores from all materials tested except painted concrete block. (At a 60-min contact time at 20 °C, log reduction observed for decorative laminate was slightly less than 6 log [5.59]).

Table 5-16. *B. anthracis* Ames Spores CFU after Various Oxonia Active® Contact Times

Material	*B. anthracis* (Ames) CFU Applied to Coupons‡	Mean CFU (SD) Recovered from Positive Control Coupons		
Glass	2.80×10^8	1.18×10^8 (1.30×10^7)		
Painted Concrete	2.30×10^8	1.01×10^8 (5.57×10^7)		
Industrial Carpet	2.30×10^8	$8.43 \times 10^{7\dagger}$ (1.40×10^7)		
Galvanized Metal	6.90×10^8	3.62×10^7 (1.70×10^7)		
Particle Board	1.50×10^8	1.22×10^8 (1.92×10^7)		
Decorative Laminate	6.90×10^8	1.05×10^7 (2.93×10^6)		
Material	***B. anthracis* (Ames) CFU Applied to Coupons‡**	**Mean CFU (SD)* Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**30 min**	**60 min**
Glass	2.80×10^8	8.26×10^2 (1.18×10^3)	6.00×10^0 (1.34×10^1)	2.06×10^2 (3.04×10^2)
Painted Concrete	2.30×10^8	1.39×10^6 (2.70×10^6)	3.02×10^5 (6.69×10^5)	2.54×10^5 (4.77×10^5)
Industrial Carpet	2.30×10^8	2.58×10^5 (3.45×10^5)	2.80×10^3 (5.72×10^3)	6.00×10^0 (1.34×10^1)
Galvanized Metal	6.90×10^8	1.18×10^4 (2.11×10^4)	0	0
Particle Board	1.50×10^8	1.01×10^3 (1.56×10^3)	0	0
Decorative Laminate	6.90×10^8	1.94×10^2 (1.47×10^2)	8.60×10^2 (1.92×10^3)	1.46×10^2 (1.50×10^2)

*SD not calculated when no viable spores (measured as CFU) were recovered from any test coupon.
†Results based on only 3 positive control coupons (rather than 5).
‡The number of spores inoculated onto the coupons was above the specified range of 7.5×10^7 - 1.25×10^8).

Table 5-17. *B. anthracis* Ames and *B. subtilis* Spores CFU after Various Oxonia Active® Contact Times‡

Material	*B. anthracis* (Ames) CFU Applied to Coupons	Mean CFU (SD) Recovered from Positive Control Coupons		
Glass	2.80×10^8	1.18×10^8 (1.30×10^7)		
Material	***B. anthracis* (Ames) CFU Applied to Coupons**	**Mean CFU (SD) Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**20 min**	**30 min**
Glass	2.80×10^8	8.26×10^2 (1.18×10^3)	6.00×10^0 (1.34×10^1)	2.06×10^2 (3.04×10^2)
Material	***B. subtilis* CFU Applied to Coupons**	**Mean CFU (SD) Recovered from Positive Control Coupons**		
Glass	2.40×10^8	$1.40 \times 10^{8‡}$ (7.07×10^6)		
Material	***B. subtilis* CFU Applied to Coupons**	**Mean CFU (SD) Recovered from Coupons After Decontamination at Specified Contact Time**		
		10 min	**20 min**	**30 min**
Glass	2.40×10^8	$2.00 \times 10^{1‡}$ (4.47×10^1)	$9.40 \times 10^{1‡}$ (1.57×10^2)	$9.80 \times 10^{2‡}$ (9.63×10^2)

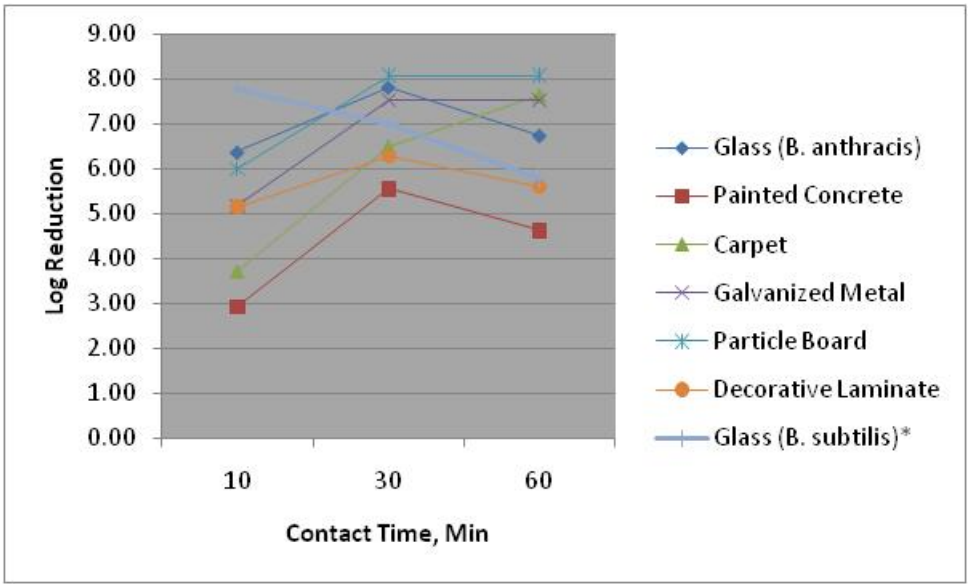
‡The procedural blank showed a low level of *B. subtilis* spores.

Table 5-18. Log Reduction in *Bacillus* Species after Various Contact Times with Oxonia Active®‡

Material	Spore	Mean Log Reduction* After Decontamination for Specified Contact Time		
		10 min	**30 min**	**120 min**
Glass	*B. anthracis* Ames	6.37	7.81	6.74
	B. subtilis	7.76‡	6.98‡	5.78‡
Painted Concrete	*B. anthracis* Ames	2.93	5.55	4.62
Carpet	*B. anthracis* Ames	3.72	6.50	7.63
Galvanized Metal	*B. anthracis* Ames	5.17	7.52	7.52
Particle Board	*B. anthracis* Ames	5.98	8.08	8.08
Decorative Laminate	*B. anthracis* Ames	5.15	6.28	5.59

*Log reduction values are calculated by taking the mean of the $\log_{10}$ recovered values of the positive control coupons and subtracting the mean of the $\log_{10}$ recovered values of the treated coupons at a given contact time with 1 CFU substituted for the observed 0 values. This procedure makes the log reduction a minimum value as indicated by the "> x" reported value.

‡The procedural blank showed a low level of *B. subtilis* spores.



*The procedural blank showed a low level of *B. subtilis* spores.

Figure 5-6. Decontamination efficacy of Oxonia Active® decontamination solution against *B. anthracis* Ames on various materials and *B. subtilis* spores on glass.

6.0 Summary

6.1 Summary of ClO_2 Fumigation Results

The ClO_2 fumigation results, summarized in Table 6-1, show efficacy against *B. anthracis* (Ames) spores, ricin toxin, and vaccinia virus.

For *B. anthracis*, the efficacy was dependent on the type of materials onto which the spores were inoculated and the strain. Shown in Table 6-1, ClO_2 fumigation at a CT of 1000 ppmv-hr (25 - 27 °C and 83 - 97% RH) resulted in no colony-forming unit (CFU) being recovered off most types of coupons (>7 log reduction). Spores on compressed cellulose insulation were the most resistant to ClO_2 fumigation, with viable spores being recovered after exposure to a CT of 9000 ppmv-hr (24 - 25 °C and 83 - 97% RH); after exposure to a CT of 12,000 ppmv-hr (25 - 27 °C and 83 - 97% RH) no viable spores were recovered from compressed cellulose insulation.

For ClO_2 fumigation, results shown in Table 6-2 suggest that increasing the temperature and humidity increased the efficacy of fumigation against *B. anthracis*.

At 24 - 27˚C, ClO_2 efficacy against *B. anthracis* on I-beam steel, bare pine wood, and ceiling tile was higher at a higher humidity (80 - 84% for ClO_2) than at a slightly lower humidity (75 - 77% for ClO_2). At high relative humidity condensation may occur. If liquid water is present, the water may dissolve ClO_2 from the air, effectively creating a liquid decontamination rather than a true fumigation.

Table 6-1. Summary of Minimum ClO_2 Fumigation CT Resulting in 0 CFU of *B. anthracis* Ames Spores, <10% Bioactivity of Ricin Applied, or 0 PFU of Vaccinia Being Recovered

Agent	Material	[ClO_2], Contact time, and CT	Temperature	RH	Biological Agent Recovery Results and Efficacy
B. anthracis (Ames) spores (1×10^8 spores applied)	Painted Concrete Galvanized Metal Decorative Laminate Particle Board Industrial Carpet Plate Glass Glass (small with only 1×10^7 spores applied)	3000 ppmv, 20 min, 1000 ppmv-hr	25 - 27 °C	83 - 97%	0 CFU extracted from any coupon Log reduction: >7 (>6 for small glass)
B. anthracis (Ames) spores (1×10^8 CFU applied)	Cellulose Insulation	3000 ppmv, 4 hr, 12,000 ppmv-hr	25 - 27 °C	83 - 97%	0 CFU extracted from any coupon Log reduction: >7
Ricin toxin (25 µg applied)	Painted Concrete Galvanized Metal Decorative Laminate Cellulose Insulation Particle Board Industrial Carpet Glass (small)	200 ppmv, 30 min, 100 ppmv-hr	23 - 25 °C	80 - 84%	93.4 - 100.0% reduction compared to controls
Vaccinia virus (2.04×10^6 - 1.52×10^8 PFU applied)	Painted Concrete Galvanized Metal Decorative Laminate Cellulose Insulation Particle Board Industrial Carpet Glass	250 ppmv, 30 min, 125 ppmv-hr	22 - 22 °C	75 - 78%	0 PFU extracted from any coupon Log reduction: >4 to >5 (depending on recovery efficiencies)

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Table 6-2. *Bacillus anthracis* Ames Spores Fumigated with ClO_2

Material	Temperature, RH	[ClO_2], ppmv	Decontaminated Coupons		
			Contact time, 0 CFU or Viable Spores	Contact time, 0 CFU or Viable Spores	Contact time, 0 CFU or Viable Spores
Glass (small)	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, 0 CFU	NA
Painted Concrete	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, 0 CFU (4 of 5 coupons)	NA
Galvanized Metal	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, 0 CFU	NA
Decorative Laminate	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, 0 CFU	NA
Particle Board	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, Invalid data	NA
Industrial Carpet	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, 0 CFU	NA
Plate Glass	24 - 25 °C 83 - 97%	3000	0.33 hr, 0 CFU	3 hr, 0 CFU	NA
Cellulose Insulation	24 - 25 °C 83 - 97%	3000	0.75 hr, 0 CFU	3 hr, Viable spores	4 hr, 0 CFU
Painted Concrete	24 - 25 °C 81 - 87%	1500	1.5 hr, 0 CFU	3 hr, 0 CFU	6 hr, 0 CFU
Painted Concrete	24 - 25 °C 72 - 82%	750	3 hr, 0 CFU	6 hr, 0 CFU	12 hr, 0 CFU
Glass (small)	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Painted Concrete	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Galvanized Metal	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Decorative Laminate	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Particle Board	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Industrial Carpet	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Plate Glass	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA
Cellulose Insulation	30 - 32 °C 74 - 89%	3000	NA	3 hr, 0 CFU	NA

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Shown in Table 6-3, ricin was reduced by 93% or greater from all building materials at a ClO_2 fumigation CT of 100 – 300 ppmv-hr at 23 °C-25 °C and 80% -84% RH.

Table 6-3. Ricin Fumigated with ClO_2

Material	Contact Time, min	[ClO_2], ppmv	Temperature	RH	% Reduction
Glass (small)	20	1500	23 - 25 °C	80 - 84%	99.84
Painted Concrete	20	1500	23 - 25 °C	80 - 84%	99.96
Galvanized Metal	20	1500	23 - 25 °C	80 - 84%	99.46
Decorative Laminate	20	1500	23 - 25 °C	80 - 84%	99.13
Cellulose Insulation	20	1500	23 - 25 °C	80 - 84%	92.74
Particle Board	20	1500	23 - 25 °C	80 - 84%	99.46
Industrial Carpet	20	1500	23 - 25 °C	80 - 84%	99.61
Glass (small)	30	200	23 - 25 °C	80 - 84%	99.75
Painted Concrete	30	200	23 - 25 °C	80 - 84%	99.97
Galvanized Metal	30	200	23 - 25 °C	80 - 84%	98.49
Decorative Laminate	30	200	23 - 25 °C	80 - 84%	99.69
Cellulose Insulation	30	200	23 - 25 °C	80 - 84%	93.42
Particle Board	30	200	23 - 25 °C	80 - 84%	95.81
Industrial Carpet	30	200	23 - 25 °C	80 - 84%	98.31

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

Shown in Table 6-4, no viable vaccinia virus was recovered from any building material tested after a ClO_2 fumigation CT of 125 – 300 ppmv-hr at 22 - 24 °C and 75 - 83% RH.

Table 6-4. Vaccinia Virus Fumigated with ClO_2

Material	Contact Time, min	[ClO_2], ppmv	Temperature, °C	RH, %	Decontaminated Coupons 0 PFU or Viable Virus
Glass (small), Painted Concrete, Galvanized Metal, Decorative Laminate, Cellulose Insulation, Particle Board, Industrial Carpet	20	1500	23 - 24	80 -83	0 PFU
Glass (small), Painted Concrete, Galvanized Metal, Decorative Laminate, Cellulose Insulation, Particle Board, Industrial Carpet	30	250	22 - 22	75-78	0 PFU

Note [ClO_2] is concentration of ClO_2 in the chamber atmosphere, in ppmv.

6.2 Summary of MeBr Fumigation Results

Shown in Figures 6-1 and 6-2, MeBr fumigation demonstrated up to complete kill (>6 log reduction, no viable spores recovered) of *B. anthracis*, dependent on the building material, the concentration of the fumigant, and the contact time.

Figure 6-1 shows that, for a given CT, a low concentration (53 mg/L) and higher contact time was generally less efficacious than a higher concentration (105, 212, or 320 mg/L) with a lower contact time for decontamination of *B. anthracis* Ames spores from glass or ceiling tile coupons.

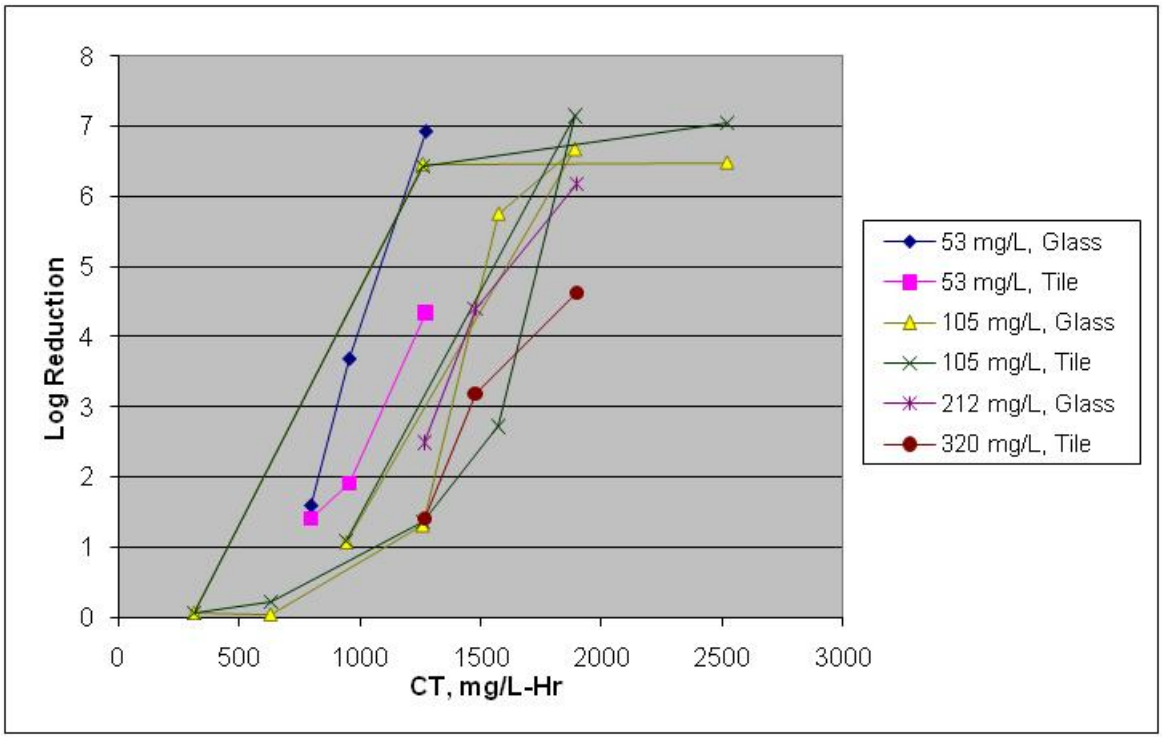


Figure 6-1. Log reduction of *B. anthracis* Ames spores vs. MeBr CT (concentration x time) at various MeBr concentrations and at 36 °C and 75% RH.

In Figure 6-2, the MeBr concentration was 105 mg/L for all fumigations shown except that 211 mg/L was used for the 1899 mg/L-hr (highest CT value). These data show that the CT required for a six log reduction in recoverable spores (measured as CFU) depends on the type of building material. Figure 6-2 also shows a comparison of MeBr decontamination efficacy for *B. anthracis* Ames and *B. subtilis* spores at various CT values. Equivalent numbers of spores (~10^7 spores) of each species were applied to identical small glass coupons as single droplets of about 10 µL each. The coupons were placed in a test chamber and fumigated with MeBr at various CTs. Little or no efficacy of MeBr against *B. subtilis* was observed in the CT range or 1260–1899 mg/L-hr. In contrast, a high level of MeBr efficacy was observed against *B. anthracis* Ames spores at CTs of 1575 mg/L-hr or greater; no viable *B. anthracis* spores

were recovered at CTs of 1890 mg/L-hr (105 mg/L for 18 hr) or 1899 mg/L-hr (211 mg/L for 9 hr).
For all MeBr fumigation trials, BIs (*B. atrophaeus* on stainless steel in Tyvek® packaging (Apex Labs)) were included. At all CTs investigated, viable *B. atrophaeus* spores were detected in the qualitative viability test; fumigation did not kill all of the *B. atrophaeus* spores inside of the Tyvek® packaging. These results are consistent with the findings for the increased resistance of *B. subtilis* to MeBr.

MeBr showed efficacy to be impacted by the RH of the test chamber. Specifically, higher humidity (75%) exhibited higher efficacy than lower humidity (40%).

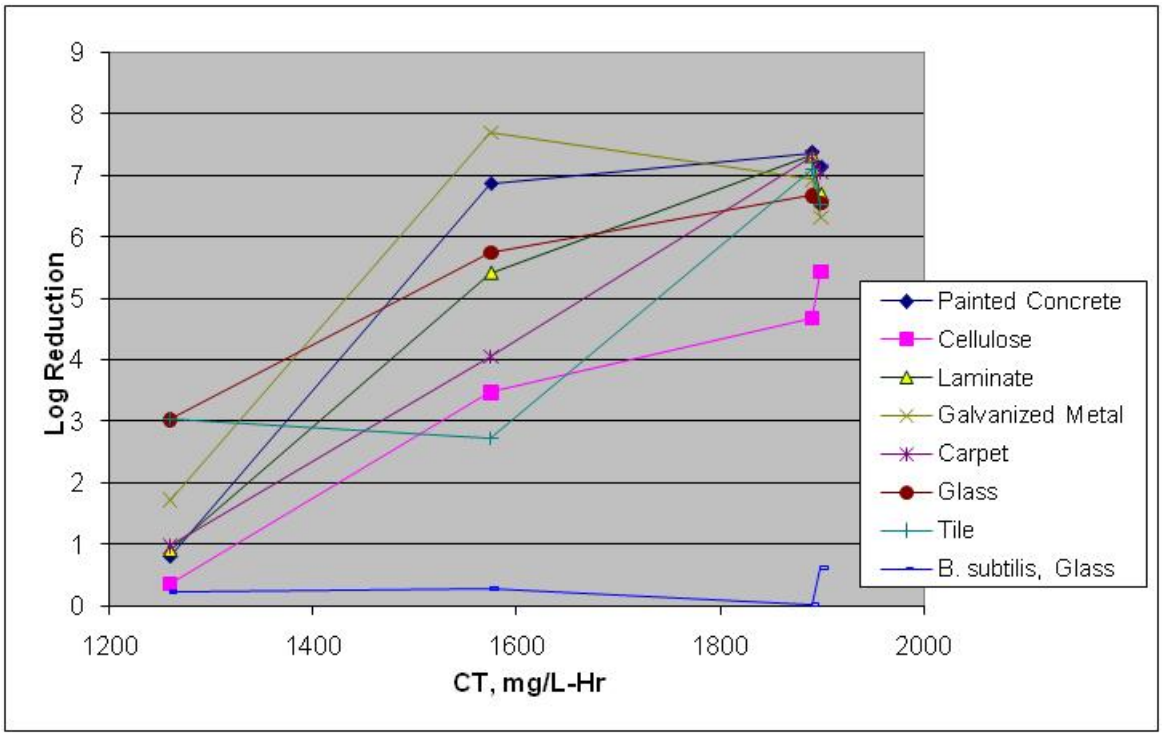


Figure 6-2. Log reduction of *B. anthracis* Ames and *B. subtilis* spores vs. MeBr CT at various MeBr concentrations at 36 °C and 75% RH.

6.3 Summary of HP Fumigation Results

Shown in Figure 6-3, HP fumigation of building materials contaminated with *B. anthracis* resulted in efficacies up to >6 log reduction (no CFU) dependent on the building material, the concentration of the fumigant, and the contact time. The HP concentration was 500 ppmv for all fumigations shown. After a 4-hr fumigation at 500 ppmv, about a 6 log reduction or higher was observed from all building materials tested. Note that HP at 250 ppmv for 1.5 hr (CT of only 375 ppmv-hr) is the treatment specified on the STERIS Vaprox® HP sterilant package insert for sporicidal efficacy (sterilization) for pre-cleaned enclosures. Note that the registration is for the solution that is used to generate the vapor.

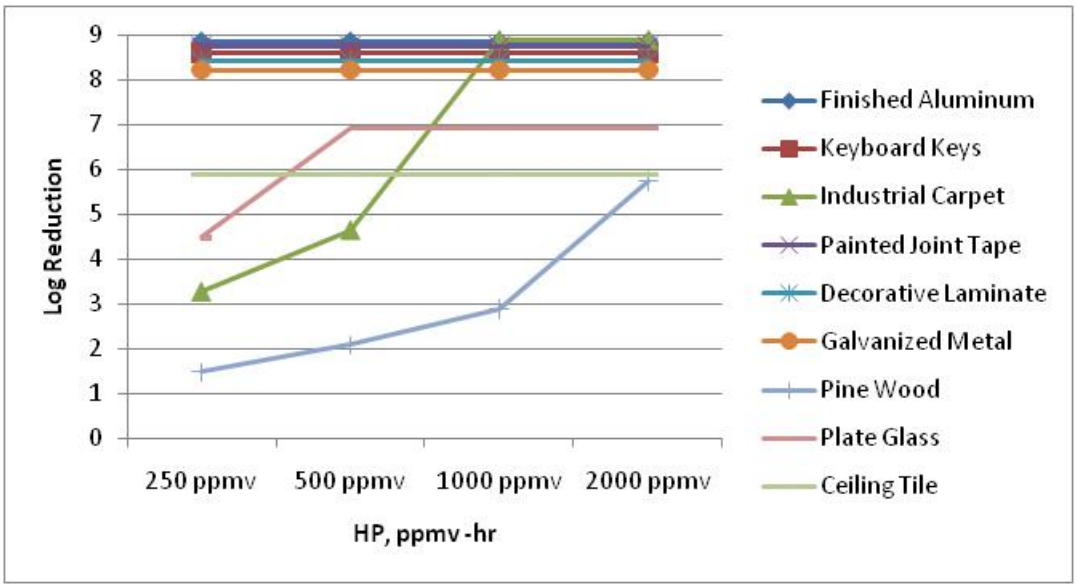


Figure 6-3. Log reduction of *B. anthracis* Ames spores vs. HP CT at 500 ppmv for various contact times.

6.4 Summary of Results from Decontamination of *B. anthracis* with Liquid pH-Amended Bleach (10% adjusted to approximately pH 7)

Shown in Figure 6-4, pH-amended bleach adjusted to pH 7 provided complete kill of *B. anthracis* Ames spores (0 CFU recovered, >6 log reduction) dependent on the building material and the contact time. Note, previous EPA testing with pH-amended bleach was also found to be effective (>7 log reduction) against *B. anthracis* spores on painted cinder block when applied as a spray (60-min contact time).[16]

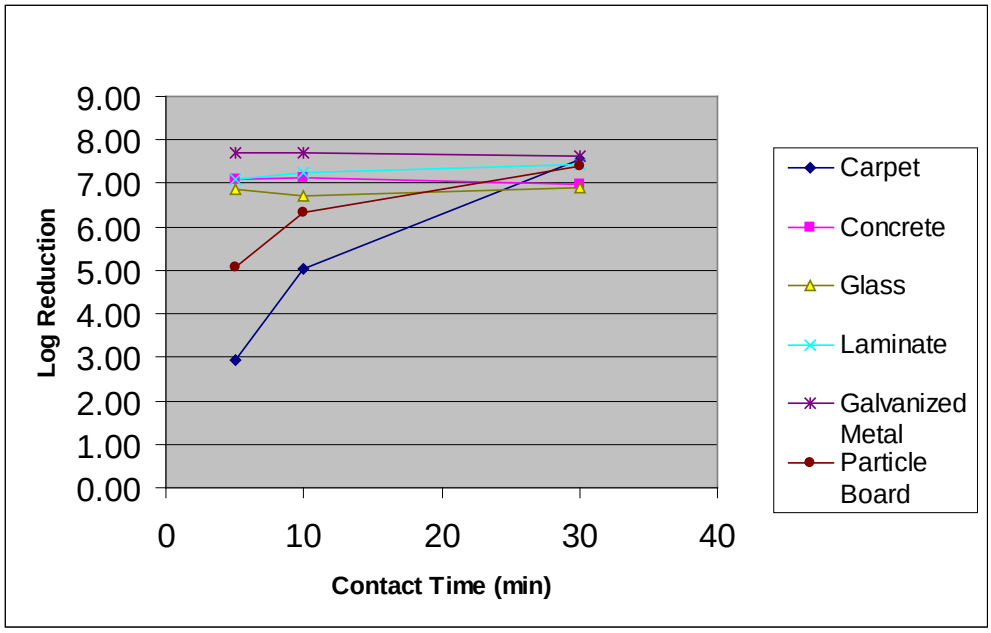


Figure 6-4. Decontamination Efficacy of pH-amended Bleach against *B. anthracis* Ames spores at 22 °C.

The efficacy of pH-amended bleach against three strains of *B. anthracis* spores (virulent Ames, avirulent NNR1Δ1, and virulent Vollum) and *B. subtilis* was evaluated using the quantitative method. The log reduction in recovery of various types of viable *Bacillus* spores on coupons exposed to pH-amended bleach or PBS for specified time periods is shown in Figure 6-5. In addition, the extracted coupons were placed individually into TSB and incubated for seven days to look for cloudiness that would indicate the presence of residual viable spores on the test coupon. In all cases where no spores were observed using the quantitative methodology, no residual viable spores were detected using the qualitative method.

Efficacy was shown to be dependent on the type of material onto which the spores were applied. For all spore types tested, viable spores were present on carpet and particle board after exposure to pH-amended bleach for five min or longer. In contrast, for all spore types tested, no viable spores were recovered from glass, galvanized metal, or decorative laminate after exposure to pH-amended bleach for five min. While strong similarities in efficacy were observed across spore types, differences were also observed. For example, viable *B. anthracis* Vollum and *B. subtilis* spores were present on painted concrete after exposure to pH-amended bleach for five min; the other two strains of *B. anthracis*, Ames and NNR1Δ1, did not have viable spores present after exposure to pH-amended bleach for five min. As another example of observed differences, for coupons of all materials tested, after 30 min exposure of *B. anthracis* Ames spores to pH-amended bleach there were no viable spores recovered from any type of material. In contrast, viable *B. subtilis* spores were recovered from both carpet and particle board after 30 min exposure to pH-amended bleach.

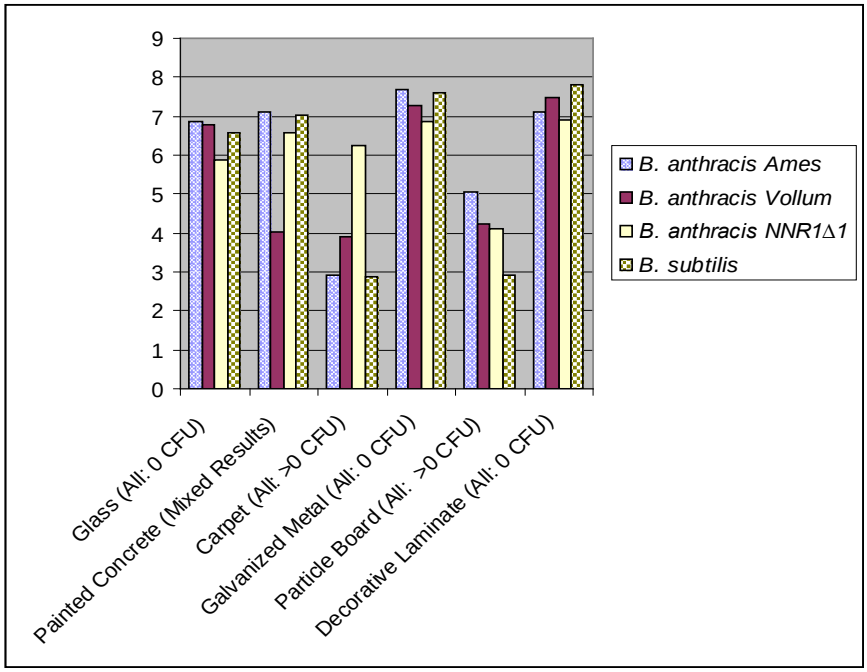


Figure 6-5. Decontamination efficacy of pH-amended bleach against various *B. anthracis* strains and *B. subtilis* at 22 °C.

6.5 Summary of Results from Decontamination of *B. anthracis* with Liquid Exterm ClO_2 Solution

Exterm ClO_2 solution provided >6 log reduction in recovered viable *B. anthracis* Ames spores dependent on the building material and the contact time (shown in Figure 6-6). No viable *B. anthracis* Ames spores were recovered from industrial carpet, decorative laminate, or glass after 120 min exposure to the ClO_2 solution; viable *B. anthracis* Ames spores were recovered from particle board after 120 min exposure to ClO_2 solution. Shown in Figure 6-7, Exterm ClO_2 solution provided >6 log reduction in recovered viable *B. subtilis* spores (no viable spores recovered) after 120 min exposure to ClO_2 solution. Viable *B. subtilis* spores were recovered from both carpet and particle board after 120 min exposure to ClO_2 solution. In general, *B. subtilis* spores appear to be able to survive a longer exposure to ClO_2 solution than *B. anthracis* Ames spores.

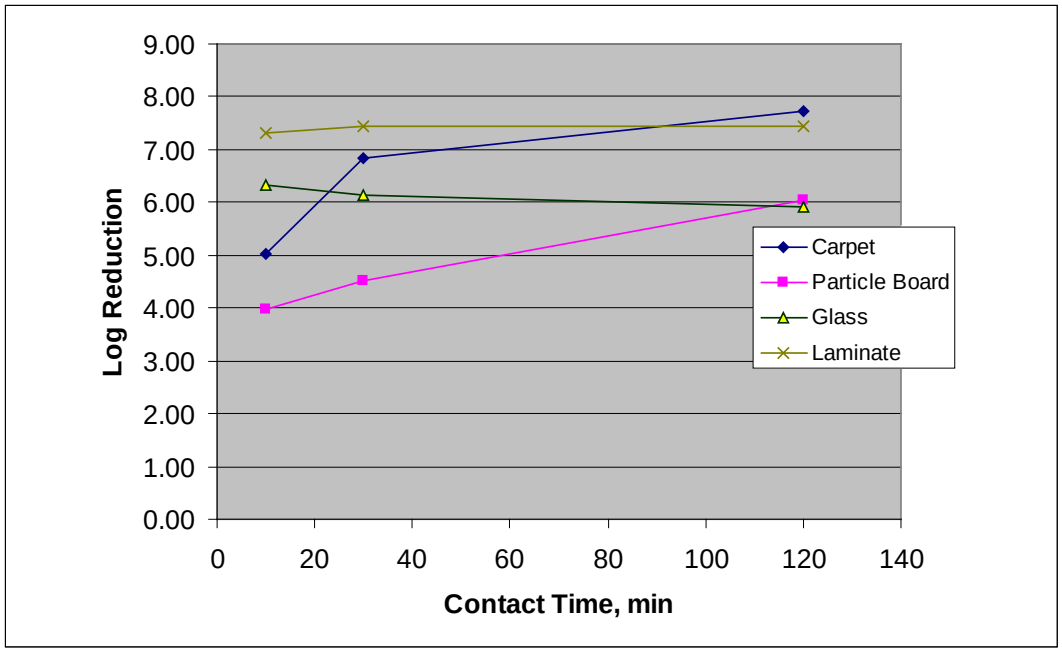


Figure 6-6. Decontamination efficacy of Exterm ClO_2 solution against *B. anthracis* Ames spores at 22 °C.

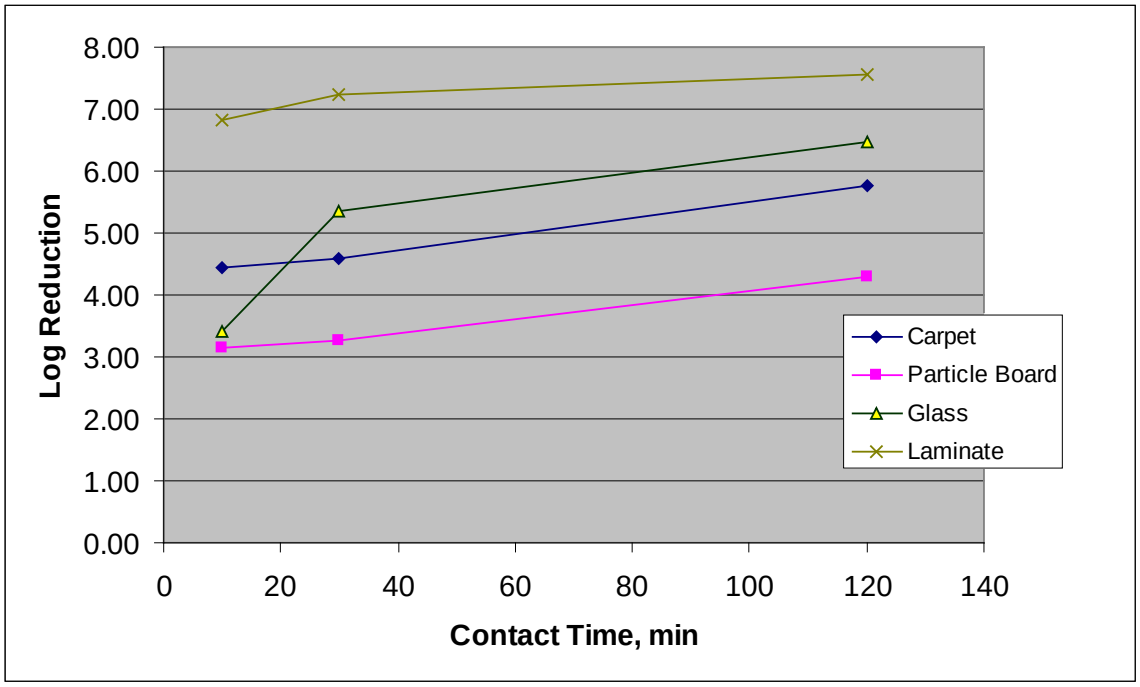


Figure 6-7. Decontamination efficacy of Exterm ClO_2 solution against *B. subtilis* spores at 22 °C.

6.6 Summary of Results from Decontamination of *B. anthracis* with Liquid Spor-Klenz® HP-PA Solution

Spor-Klenz® HP-PA solution exhibited a range of efficacies, up to >6 log reduction in *B. anthracis* Ames spores (no viable spores recovered), dependent on the building material and contact time (shown in Figure 6-8). No viable *B. anthracis* Ames or *B. subtilis* spores were recovered from any glass coupons after a 20-30 min contact time. A complete kill of spores on decorative laminate, i.e., no viable spores recovered or detected, occurred at a 30-min contact time with Spor-Klenz® HP-PA for *B. anthracis* Ames spores. In contrast, less than a 2-log reduction in viable spores was observed after a 30-min contact time with Spor-Klenz® HP-PA for *B. anthracis* Ames spores on galvanized metal. Spor-Klenz® HP-PA efficacy was greater against *B. anthracis* Ames spores on carpet than on metal, but after a 30-min contact time with Spor-Klenz® HP-PA less than a 4-log reduction was observed.

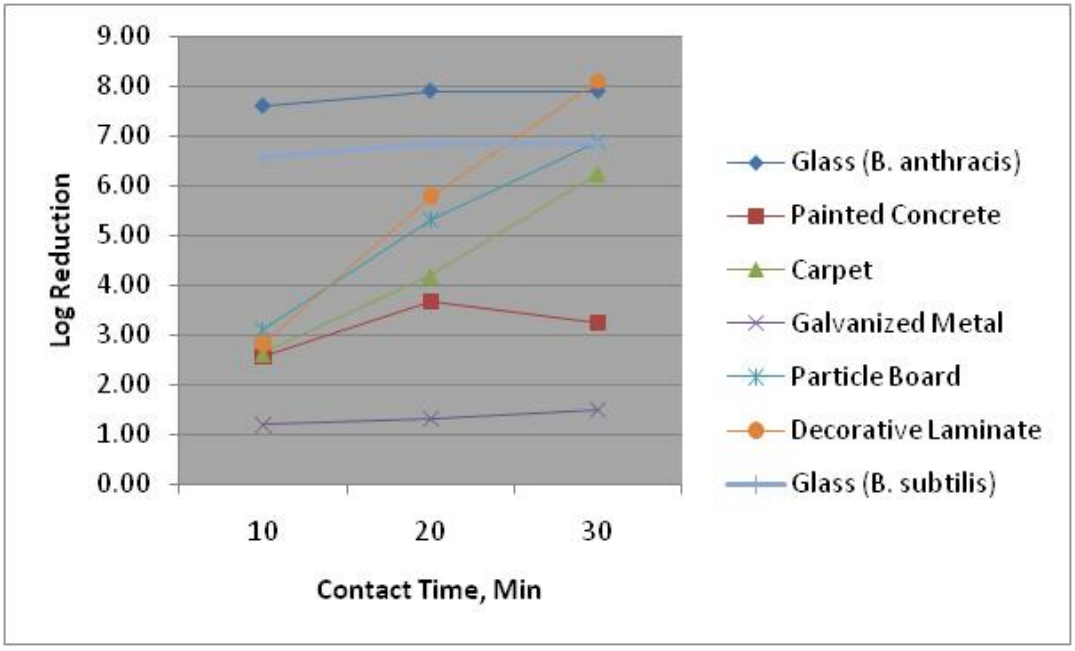


Figure 6-8. Decontamination efficacy of Spor-Klenz® HP-PA decontamination solution against *B. anthracis* Ames on various materials and *B. subtilis* spores on glass.

6.7 Summary of Results from Decontamination of *B. anthracis* with Liquid Oxonia Active®

Oxonia Active® exhibited a range of efficacies, up to >6 log reduction in *B. anthracis* Ames spores (no viable spores recovered), dependent on the building material and contact time (shown in Figure 6-9). No *B. anthracis* Ames spores were recovered from any galvanized metal or particle board coupons after a 30- or 60-min contact time. Viable *B. anthracis* spores were recovered from one or more coupons of glass, painted concrete, carpet, and

decorative laminate after a 60-min contact time with Oxonia Active®. The efficacy of Oxonia Active® against *B. anthracis* Ames and *B. subtilis* spores, reported as log reduction, showed similar efficacies. For both *B. anthracis* Ames and *B. subtilis* spores, a >6 log reduction was observed after a 10-min contact time (except that only a 5.77 log reduction was observed for *B. subtilis* after 60 min). Small numbers of viable spores were recovered from one or more replicate coupons for both *B. anthracis* Ames and *B. subtilis* spores at each contact time (10, 30, and 60 min).

Decontamination using Oxonia Active® against *B. anthracis* Ames and *B. subtilis* spores, reported as log reduction, showed similar efficacies. For both *B. anthracis* Ames and *B. subtilis* spores, a >6 log reduction was observed after a 10-min contact time. Small numbers of viable spores were recovered from one or more replicate coupons for both *B. anthracis* Ames and *B. subtilis* spores at each contact time (10, 20, and 30 min).

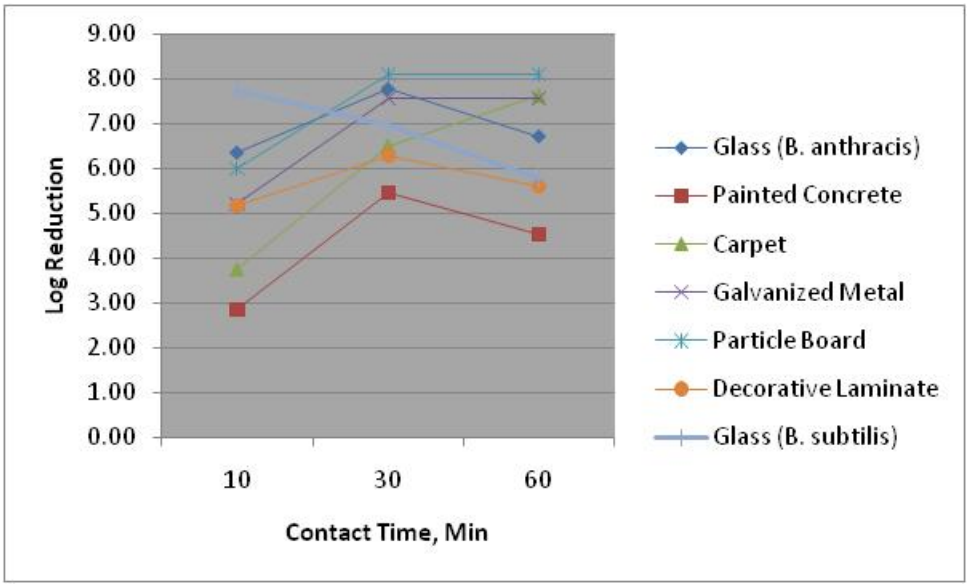


Figure 6-9. Decontamination efficacy of Oxonia Active® against *B. anthracis* Ames on various materials and *B. subtilis* spores on glass.

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