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Methyl Bromide Decontamination of Indoor and Outdoor Materials Contaminated with *Bacillus anthracis* Spores

Office of Research and Development
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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

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Executive Summary

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), is striving to protect human health and the environment from adverse impacts resulting from the intentional release of threat agents. As part of this mission, the EPA's Homeland Security Research Program (HSRP) is investigating the effectiveness and applicability of technologies for homeland security (HS)-related applications. This report provides the results of an assessment to determine the decontamination efficacy of methyl bromide (MeBr) fumigant in inactivating *Bacillus anthracis* (*B.a.*; causative agent for anthrax) spores on indoor and outdoor materials. In particular, to facilitate future decontaminations employing MeBr, this investigation focused on finding efficacious conditions when using MeBr at temperatures and relative humidity (RH) levels lower than used in previous studies. Another objective of the study was to compare these results with other spore-forming microorganisms to assess their potential as a representative surrogate for *B.a.* Ames, for use in field studies and additional lab-based investigations.

This investigation focused on the decontamination of six types of common indoor and outdoor materials: glass, ceiling tile, carpet, painted wallboard paper, bare pine wood, and unpainted concrete. Decontamination efficacy tests were conducted with spores of virulent *B.a.* Ames and the non-virulent strains *Geobacillus stearothermophilus* [*G.s.*], *B.a.* NNR1Δ1, and *B.a.* Sterne. Decontamination efficacy was quantified in terms of log reduction (LR), based on the difference in the number of bacterial spores recovered from positive control coupons and test coupons. Tests were conducted at varying temperatures, RH levels, MeBr concentrations, and contact times to assess the effect of these operational parameters on decontamination efficacy. Twenty tests were conducted with MeBr, with target concentrations of either 212 or 300 milligrams per liter (mg/L). Additionally, the target temperature during testing ranged from 22 to 32 °C, the target RH was either 45 or 75 %, and contact times ranged from 18 to 72 hours.

Summary of Results

As seen in other similar fumigant evaluations[1], the concentration, temperature, RH, and contact time affect the efficacy of MeBr against *B.a.* Ames. Table E-1 shows the contact time required to achieve >6 LR (a decontaminant that achieves an LR value >6 is considered effective)[2] on all materials tested for a given set of fumigation conditions (MeBr level, temperature, and RH). For example, a contact time of 36 hours was required to achieve > 6 LR of *B.a.* Ames on all materials when fumigating at 212 mg/L, 22 °C, and 75 % RH. However, only 18 hours of contact time were required to achieve > 6 LR of *B.a.* Ames on all materials when the MeBr concentration was increased to 300 mg/L and temperature increased to 27 °C.

The test program originally began using two microorganisms and the six aforementioned test materials in each experiment. But to evaluate three microorganisms at once, the number of coupon materials for each experiment was reduced from six to four, due to the size of the MeBr test chamber. Painted wallboard and unpainted concrete were removed from the latter part of the study, as these two material types generally exhibited higher decontamination efficacy than the other materials. In contrast, test results showed that glass and wood were the materials most difficult to decontaminate (exhibited lower efficacy than the other four material types).

The data generated from this investigation show that *G.s.* is less resistant than *B.a.* Ames (*G.s.* was inactivated at a higher average LR than *B.a.* Ames) in the two tests conducted with *G.s.* In every test conducted with the NNR1Δ1 strain, the NNR1Δ1 strain was always more resistant than the Ames strain. (In a few of the tests using the NNR1Δ1 strain, the average difference in LR with the Ames strain was more than 6.0.) In the tests with the Sterne strain, Sterne was

always inactivated to a higher degree than the Ames strain when fumigating at 45 % RH. But when fumigating at 75 % RH, the Sterne strain was more resistant than *B.a.* Ames.

This study shows the important role that RH plays when fumigating with MeBr. There were no tests in which >6 LR of *B.a.* Ames was achieved on all materials when fumigating at 45 % RH. When fumigating at 45 % RH, increasing the MeBr concentration, temperature, or contact time generally did not improve decontamination efficacy. In contrast, when fumigating at 75 % RH, increasing the MeBr concentration, temperature and contact time did generally improve efficacy.

Table E-1. Contact Time Required to Achieve >6 LR on all Materials*

Target MeBr Concentration (mg/L)	Target Temperature (° C)	Target RH (%)	Time (hours) Required to Achieve >6 LR on All Materials[c]				Test Number Reference[a]
			B.a. Ames	G.s.	*B.a.* NNR1Δ1	*B.a.* Sterne	
212	22	45	> 60	48	> 60	> 60	1, 2, 11
212	22	75	36	--[b]	> 36	--	3, 5
212	27	45	> 48	--	> 36	> 48	4, 6, 8
212	27	75	36	--	> 36	36	7, 9
212	32	45	> 72	--	> 72	> 72	13, 16, 19
212	32	75	24	--	> 24	24	12,
300	22	45	> 60	--	> 60	> 60	10, 15
300	22	75	24	--	> 24	> 24	14
300	27	45	> 60	--	> 60	> 60	18
300	27	75	18	--	> 18	> 18	17
300	32	45	> 60	--	> 60	> 60	20

* Materials tested were glass, ceiling tile, carpet, painted wallboard paper, bare pine wood and unpainted concrete.
[a] Detailed data from each test number can be referenced in Tables A-1 through A-4 in Appendix A.
[b] "--" Not tested.
[C] > indicates that >6 LR on all materials was not achieved at the contact time listed, and contact time was the longest tested.

Impact of Study

This research provides information on the efficacy of MeBr fumigation to decontaminate materials that have been contaminated with *B. anthracis* spores. Such results may be useful in the development of guidance to aid in deployment of MeBr fumigation after a wide-area release of *B. anthracis* spores. In particular, these results will provide decision makers with information for effectively using MeBr at temperatures and RH levels lower than has been recommended previously, which will facilitate its use. This report also provides data to assist in selection of an avirulent surrogate for *B. anthracis* Ames when using MeBr, for use in future field studies and additional lab-based investigations.

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Abbreviations/Acronyms

B.a.	*Bacillus anthracis*
BBRC	Battelle Biomedical Research Center
BOTE	Bio-Response Operational Testing and Evaluation
BSC	biological safety cabinet
CFU	colony-forming unit(s)
CI	confidence interval
cm	centimeter(s)
°C	degrees Celsius
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
ft	foot/feet
G.s.	*Geobacillus stearothermophilus*
HCl	hydrochloric acid
HS	homeland security
HSRP	Homeland Security Research Program
kGy	kilogray
L	liter(s)
LAL	Limulus Amebocyte Lysate (assay)
LED	light emitting diode
LR	log reduction
MeBr	methyl bromide
min	minute(s)
mg	milligram(s)
mL	milliliter(s)
µL	microliter(s)
NA	not applicable
NHSRC	National Homeland Security Research Center
NT	not tested
ORD	EPA Office of Research and Development
oz	ounce(s)
PBST	phosphate-buffered saline + 0.1% Triton X-100
PCR	polymerase chain reaction
ppm	part(s) per million
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RH	relative humidity
rpm	revolution(s) per minute
SD	standard deviation
SE	standard error
SFW	sterile filtered water (cell-culture grade)
TSA	technical systems audit(s)

1.0 Introduction

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) is helping protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, the HSRP is working to develop tools and information that will help detect the intentional introduction of chemical or biological contaminants into buildings or water systems; contain these contaminants; decontaminate buildings, outdoor environments, or water systems; and facilitate the disposal of material resulting from restoration activities.

In this work, the efficacy of methyl bromide (MeBr) against *Bacillus anthracis* (*B.a.*) Ames and candidate surrogate spores applied to indoor and outdoor materials (glass, ceiling tile, carpet, painted wallboard paper, bare pine wood and unpainted concrete) was investigated. The study builds on previous laboratory research conducted by EPA to assess decontamination efficacy of MeBr for inactivating *B. anthracis* spores on different materials[1-3]. In addition, MeBr was used in Phase 2 of the full-scale Bio-Response Operational Testing and Evaluation (BOTE) project. At the BOTE Phase 2 demonstration, issues related to achieving the target fumigation conditions were encountered, i.e., there were difficulties in achieving the relatively high target fumigation temperature, humidity, and MeBr concentration in the facility. One objective of this study was, therefore, to find effective fumigation conditions at relatively lower temperature and/or RH conditions (i.e., conditions that would not require supplementary heating or humidification), which would facilitate fumigation with MeBr.

Lastly, another objective of this work was to obtain side-by-side efficacy data for *B.a.* Ames and other microbes that could be used to assess the suitability of candidate surrogates for *B. a.* Ames when decontaminating with MeBr. Previous tests[1-4] with *B. atrophaeus* or *B. subtilis* showed these species to be overly resistant to MeBr compared with *B.a.* Ames. The Ames strain of *B.a.* was chosen for testing because the Ames strain of *B.a.* was the strain identified in the Amerithrax incident in 2001[7].

2.0 Technology Description and Test Matrices

2.1 Technology Description

MeBr (Chemtura, Philadelphia, PA) has been registered by the EPA for soil fumigation (injected into the soil before a crop is 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). Although MeBr has also been demonstrated to be an effective biocide against *B. anthracis* on building materials and soil,[1-3] the focus of this study was to determine effective conditions at lower RH levels and/or temperatures, thereby making MeBr fumigation for *B.a.* easier to implement. Furthermore, although MeBr use is being phased out under the Montreal Protocol, MeBr is still currently and widely used via critical use exemptions as a soil and commodity (quarantine) fumigant.[6]

2.2 Test Matrix

The test matrix for the MeBr fumigation tests is shown in Table 2-1. As testing proceeded, adjustments were made to one of the fumigation parameters (contact time, temperature, RH, concentration) to assess the effect of that parameter and to find efficacious conditions. The first eight tests were conducted with all six materials, using *B.a* Ames and one other bacterium. In Tests 9-20, two materials were eliminated from testing (unpainted concrete and painted wallboard paper) to allow for the simultaneous testing of three microorganisms. In the latter 12 tests, testing focused on *B.a.* Ames, Sterne, and NNR1Δ1, and *G.s.* was no longer tested. Unpainted concrete and wallboard paper were removed from the latter phase of testing as decontamination efficacy was the highest for these materials. Tests 6 and 8 utilized the same operational parameters to assess repeatability.

Table 2-1. MeBr Test Matrix

Test Number	Materials	Microorganisms	Target Fumigation Parameters			Contact Time (hours)
			MeBr Concentration (mg/L)	Temperature (°C)	RH (%)	
1	Glass Ceiling Tile Carpet Painted Wallboard Paper Bare Pine Wood Unpainted Concrete	*B. anthracis* Ames *G. stearothermophilus*	212	22	45	36
2		*B. anthracis* Ames *G. stearothermophilus*	212	22	45	48
3		*B. anthracis* Ames *B. anthracis* NNR1Δ1	212	22	75	36
4		*B. anthracis* Ames *B. anthracis* NNR1Δ1	212	27	45	36
5		*B. anthracis* Ames *B. anthracis* NNR1Δ1	212	22	75	24
6		*B. anthracis* Ames *B. anthracis* Sterne	212	27	45	48
7		*B. anthracis* Ames *B. anthracis* Sterne	212	27	75	24
8		*B. anthracis* Ames *B. anthracis* Sterne	212	27	45	48
9	Glass Ceiling Tile Carpet Bare Pine Wood	*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	212	27	75	36
10		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	300	22	45	48
11		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	212	22	45	60
12		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	212	32	75	24
13		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	212	32	45	48
14		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	300	22	75	24
15		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	300	22	45	60
16		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	212	32	45	60
17		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	300	27	75	18
18		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	300	27	45	60
19		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	212	32	45	72
20		*B. anthracis* Ames *B. anthracis* NNR1Δ1 *B. anthracis* Sterne	300	32	45	60

3.0 Test Procedures

This section provides an overview of the procedures that were used for the bench-scale evaluation of MeBr to inactivate *B. anthracis* Ames and potential surrogate spore species on six different materials.

3.1 Biological Agents

The virulent *B.a.* spores used for this testing were prepared from a qualified stock of the Ames strain at the Battelle Biomedical Research Center (BBRC, Lot B21, West Jefferson, OH). The spore lot was subjected to a stringent characterization and qualification process required by Battelle's standard operating procedure for spore production. Specifically, the spore lot was characterized prior to use by observation of colony morphology, direct microscopic observation of spore morphology, and size and determination of percent refractivity and percent encapsulation. In addition, the number of viable spores was determined by colony count and expressed as colony forming units per milliliter (CFU/mL). Theoretically, once plated onto bacterial growth media, each viable spore germinates and yields one CFU. Variations in the expected colony phenotypes were recorded. Endotoxin concentration of each spore preparation was determined by the Limulus Amebocyte Lysate (LAL) assay to assess whether contamination from gram-negative bacteria occurred during the propagation and purification process of the spores. Genomic deoxyribonucleic acid (DNA) was extracted from the spores and DNA fingerprinting by polymerase chain reaction (PCR) was done to confirm the genotype. This work was performed by Dr. Paul Keim at Northern Arizona University. The virulence of the spore lot was measured at Battelle by challenging guinea pigs intradermally with a dilution series of spore suspensions, and virulence was expressed as the intradermal median lethal dose. In addition, testing was conducted for robustness of the spores via hydrochloric acid (HCl) resistance.

Geobacillus stearothermophilus (*G.s.*; ATCC 12980), B.a. NNR1Δ1 (Received from Edgewood Chemical and Biological Center, Edgewood, MD), and B.a. Sterne 34f2 (Colorado Serum Company, Denver, CO) spores were tested alongside the virulent form of B.a. (Ames) to assess their potential as a surrogate. Using growth from a stock culture, G.s., B.a. NNR1Δ1, or B. a. Sterne was inoculated into 10 mL tubes of nutrient broth and incubated in a shaking incubator for 24 ± 2 hours at approximately 150 revolutions per minutes (rpm). The B.a. Ames strain was prepared using a BioFlo 3000 fermenter (New Brunswick Scientific Co., Inc, Edison, NJ). Incubation temperature for the B.a. strains was 37 degrees Celsius (°C), while for G.s., an incubation temperature of 55 °C was used. This culture was used to inoculate amended nutrient agar plates. Plates were inoculated with 500 microliters (µL) of the culture and spread with a sterile plate spreader. Plates were inverted (with no shaking) and incubated for 12-14 days. Following incubation, plates were harvested by washing with 10 mL sterile water and scraped into sterile tubes. The harvested spores were centrifuged at 5000 rpm and washed with water three times and resuspended in sterile water. The prepared spores were examined via microscopy and determined to have >95 % refractile spores with <5 % cellular debris. All stock spore suspensions were prepared in sterile filtered water (SFW) at an approximate concentration of 1 x 109 CFU/mL and stored under refrigeration at 2 to 8 °C.

3.2 Test Materials

Decontamination testing was conducted on glass, ceiling tile, carpet, painted wallboard paper, bare pine wood and unpainted concrete. Information on these materials is presented in Table 3-1, and a picture of each is presented in Figure 3-1. Material coupons were cut to uniform length and width from a larger piece of stock material. Materials were prepared for testing either by sterilization via gamma irradiation at ~40 kilogray (kGy; STERIS Isomedix Services,

Libertyville, IL) or by autoclaving at 121 °C for 15 minutes. Gamma-irradiated material coupons were sealed in 6 mil Uline Poly Tubing (Uline, Chicago, IL), and autoclaved coupons were sealed in sterilization pouches (Fisher, Pittsburgh, PA) to preserve sterility until the coupons were ready for use.

Table 3-1. Test Materials

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name	Approximate Coupon Size, width x length x thickness	Material Preparation
Glass	C1036	Brooks Brothers, Columbus, OH	1.9 centimeter (cm) x 7.5 cm x 0.3 cm	Autoclave
Ceiling Tile	Armstrong® B513, classic fine textured	Armstrong, Columbus, OH	1.9 cm x 7.5 cm x 0.3 cm	Gamma Irradiation
Carpet	Shaw Swizzle EcoWorx, Style: 10401 Color: Jacks	Shaw Industries, Dalton, GA	1.9 cm x 7.5 cm x 0.3 cm	Gamma Irradiation
Painted Wallboard Paper	Roller painted on one side using Martin Senour Paints. One primer (#71-1185) and two finish (flat, #70-1001) coats	United States Gypsum Company, Chicago, IL	1.9 cm x 7.5 cm x 0.1 cm	Gamma Irradiation
Bare Pine Wood	Generic Molding	Lowes, Columbus, OH	1.9 cm x 7.5 cm x 0.3 cm	Gamma Irradiation
Unpainted Concrete	ASTM C90 cinder block	Wellnitz Columbus, OH	1.9 cm x 7.5 cm x 0.6 cm	Autoclave

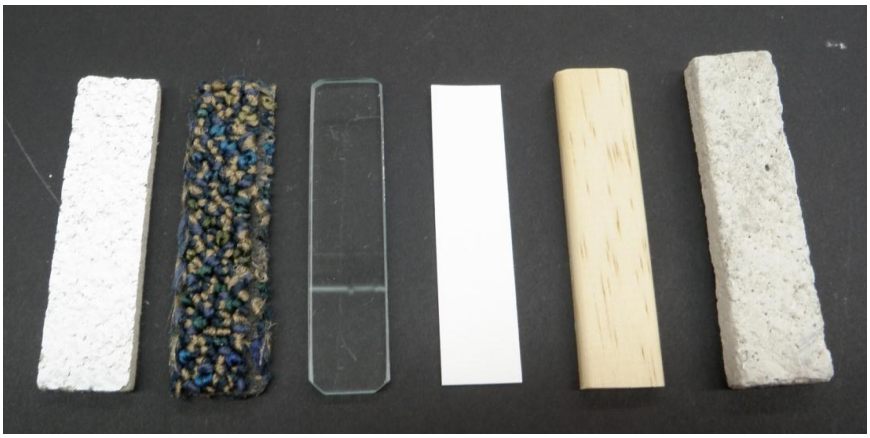


Figure 3-1. Coupon types from left to right: ceiling tile, carpet, glass, painted wallboard paper, bare pine wood, and unpainted concrete.

3.3 Preparation of Coupons

Test and positive control coupons were placed on a flat surface within a Class II biological safety cabinet (BSC) and inoculated with approximately 1 x 10^8 CFU of viable *B.a.* Ames (or indicated surrogate) spores per coupon. A 100 µL aliquot of a stock suspension of approximately 1 x 10^9 CFU/mL was dispensed using a micropipette applied as 10 µL droplets across the coupon surface (see Figure 3-2). This approach provided a more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. After inoculation, the coupons were transferred to a Class III BSC and left undisturbed overnight to dry under ambient conditions, approximately 22 °C and 40 % relative humidity (RH).

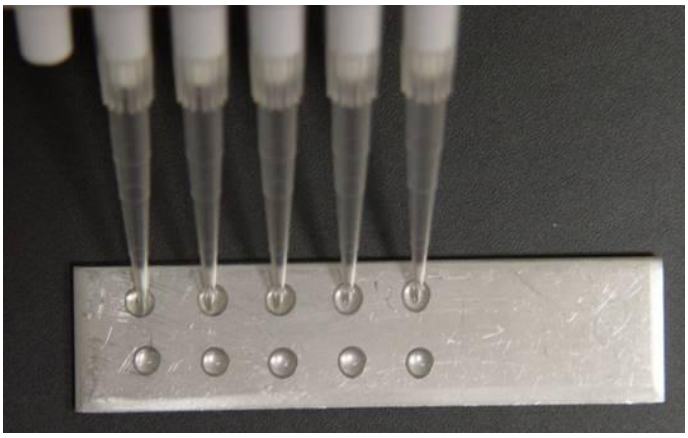


Figure 3-2. Liquid inoculation of coupon using a micropipette.

The number and type of replicate coupons used for each combination of material, decontaminant, concentration, and environmental conditions were:

- five test coupons (inoculated with *B. anthracis* or surrogate spores and exposed to decontaminant)
- five positive controls (inoculated with *B. anthracis* or surrogate spores but not exposed to decontaminant)
- one laboratory blank (not inoculated and not exposed to the decontaminant)
- one procedural blank (not inoculated and exposed to the decontaminant)

On the day following liquid spore inoculation, coupons intended for decontamination (including blanks) were transferred into a test chamber and exposed to the MeBr fumigant using the apparatus and application conditions specified in Section 4.0 of this report. Control coupons were added to the control chamber as described in Section 4.0.

3.4 Coupon Extraction and Biological Agent Quantification

For sample extraction, test coupons, positive controls, and blanks were placed in 50 mL polypropylene conical tubes containing 10 mL of sterile phosphate buffered saline + 0.1% Triton X-100 (PBST). The vials were capped, placed on their sides and agitated on an orbital shaker for 15 minutes (min) at approximately 200 rpm at room temperature.

Residual viable spores were determined using a dilution plating approach. Following extraction, the extract was removed and a series of 10-fold dilutions was prepared in sterile water. An aliquot (0.1 mL) of either the undiluted extract and/or each serial dilution were plated onto tryptic soy agar in triplicate and were incubated for 18-24 hours at 35-37 ºC. Colonies were counted manually and CFU/mL were determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Dilution data representing the greatest number of individually definable colonies were expressed as arithmetic mean ± standard deviation (SD) of the numbers of CFU observed. Laboratory blanks controlled for sterility, and procedural blanks controlled for viable spores inadvertently introduced to test coupons. The blanks were spiked with an equivalent amount of 0.1 mL of SFW. The target acceptance criterion was that extracts of laboratory or procedural blanks were to contain no CFU.

After each decontamination test, the BSC III and the MeBr test and control chambers were thoroughly cleaned (using separate steps involving bleach, ethanol, water, then drying).

3.5 Decontamination Efficacy

The mean percent spore recovery from each coupon was calculated using results from positive control coupons (inoculated, not decontaminated), by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean } CFU_{pc}/CFU_{spike}] \times 100 \qquad (1)$$

where Mean CFU_{pc} is the mean number of CFU recovered from five replicate positive control coupons of a single material, and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} is known from enumeration of the stock spore suspension. One aliquot of the stock suspension is plated and enumerated on each day of testing to confirm CFU_{spike} concentration. Spore recovery was calculated for *B.a.* Ames or surrogate on each coupon, and the results are included in Section 5 and Appendix A.

The performance or efficacy of MeBr was assessed by determining the number of viable organisms remaining on each test coupon after decontamination. Those numbers were compared to the number of viable organisms extracted from the positive control coupons.

The number of viable spores of *B.a.* Ames or surrogate in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction or LR) to which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons. The logarithm of the CFU abundance from each coupon extract was determined, and the mean of those logarithm values was then determined for each set of control and associated test coupons, respectively. Efficacy of a decontaminant for a test organism/test condition on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy\ (LR) = \overline{(\log_{10} CFUc_{ij})} - \overline{(\log_{10} CFUt_{ij})} \qquad (2)$$

where $\log_{10} CFUc_{ij}$ refers to the j individual logarithm values obtained from the positive control coupons, $\log_{10} CFUt_{ij}$ refers to the j individual logarithm values obtained from the corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five positive controls and five corresponding test coupons (i.e., $j = 5$) for each coupon. A decontaminant that achieves a 6 LR or greater is considered effective. [2]

In the case where no viable spores were found in any of the five test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a $\log_{10}$ CFU of zero for that material. This situation occurred when the decontaminant was highly effective, and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to (≥) the value calculated by Equation 2.

The variances (i.e., the square of the SD) of the $\log_{10} CFUc_{ij}$ and $\log_{10} CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 3, as follows:

$$SE = \sqrt{\frac{S^2 c_{ij}}{5} + \frac{S^2 t_{ij}}{5}} \qquad (3)$$

where the number 5 again represents the number j of coupons in both the control and test data sets. Each efficacy result is reported as a LR value with an associated 95 % confidence interval (CI), calculated as follows:

$$95\ \%\ \text{CI} = \textit{Efficacy (LR)} \pm (1.96 \times \text{SE}) \quad (4)$$

The significance of differences in efficacy across different test conditions and spore types was assessed based on the 95 % confidence interval of each efficacy result. Differences in efficacy were judged to be significant if the 95 % CIs of the two efficacy results did not overlap. Any results based on this formula are hereafter noted as significantly different. Note this comparison is not applicable when the two efficacy results being compared are both reported with LRs as ≥ some value.

The average difference in efficacy was determined when comparing the results of two tests and reported as a LR value. This difference in efficacy was calculated as follows:

$$\textit{Avg Difference in Efficacy (LR)} = \frac{\sum_{a=1}^{n} LR_{a,2} - LR_{a,1}}{n} \quad (5)$$

where the letters *a* through *n* represent the material types, the number *1* represents *B.a.* Ames, and the number *2* represents the avirulent (potential surrogate) microorganism for which results are being compared. The letter *n* represents the number of materials tested, with *n* = 6 for Tests 1-8, and equal to 4 for the remaining tests. When both values were ≥ LR (indicating complete inactivation), these were not included in the formula. A positive value indicates that the avirulent organism was inactivated on average to a higher degree (i.e., it was less resistant) across the materials tested compared to *B.a.* Ames.

In some instances, significant differences in average efficacy between tests were assessed with a t-test using Microsoft Excel®, according to the formula below:

$$t = \frac{\overline{X_1} - \overline{X_2}}{S_{\overline{X_1} - \overline{X_2}}} \quad (6)$$

where $\overline{X_1}$ and $\overline{X_2}$ are the means of Tests 1 and 2, respectively. $S_{\overline{X_1} - \overline{X_2}}$ is the standard error of the difference between Tests 1 and 2. Excel produces a p-value, a statistic calculated from the t-test, used to assess whether the averages of the two tests are reliably different from each other. Using this formula, a p-value was assigned where indicated. If the calculated p-value was <0.05, then the two sets of data were considered to be significantly different.

3.6 Repeatability

It was desired to perform a statistical evaluation to formally test for repeatability between Test 6 and 8 within each of *B.a.* Ames and *B.a.* Sterne. The limited number of decontamination data points in each test (n=6), as well as the underlying variability in decontamination results, make this comparison a challenge. To address this challenge, a statistical hypothesis test approach was developed. In this form of a statistical comparison, a statistical measure of interest is identified. Then, a null hypothesis relative to the underlying comparison is proposed along with an alternative hypothesis (usually what is desired to be shown). The statistical measure from the observed data is compared to what could have been observed. If there is adequate probabilistic evidence that the observed results are unusual, the null hypothesis can be rejected in favor of concluding the alternative.

A statistical measure to show the repeatability of the testing is the square root of the sum of the squared differences in average LR of bacteria between the first and second tests within each material.

(6)

$$Test\ Statistic = \sqrt{\sum_{m=1}^{6} \left(LR_{m,Test\ 8} - LR_{m,Test\ 6}\right)^2}$$

where *m* is the material types (glass, ceiling tile, carpet, painted wallboard paper, bare pine wood, and unpainted concrete) and $LR_{m,Test\ x}$ is the mean log reduction for material *m* in Test *x*.

Note: For results with a reduction "≥" a value, the statistic was calculated using the subsequently reported value (e.g., 7.34 for ≥7.34).

Since there is not adequate information regarding the true variability in LR at a population level, a bootstrap approach was used where the observed data serve as the source population from which sampling is done. The statistical analysis for this evaluation consisted of enumerating all the 720 permutations of how the second set of six material log reduction results could have been observed. As an example, consider for the Ames test that one possible permutation of Test 8 would have been for the observed log reductions to have been ≥6.94 for glass, 2.03 for ceiling tile, ≥7.60 for carpet, 4.31 for painted wallboard paper, 3.29 for bare pine wood, and 2.29 for painted concrete. For each of the 720 permutations, a test statistic is calculated. In the Ames example just noted, a value of 6.979 would be calculated. The actual observed test statistic (2.701 for Ames, 3.194 for Sterne) was then compared to a ranking of the entire set of 720 permutations within each agent. If the observed statistic was below the 5th percentile of the ranking, it provided at least 95 % confidence that the test results were repeatable.

3.7 Surface Damage

The physical effect of MeBr on the materials was also qualitatively monitored during the evaluation. This approach provided a gross visual assessment of whether the decontaminants altered the appearance of the test materials. The procedural blank (coupon that is decontaminated, but has no spores applied) was visually compared to a laboratory blank coupon (a coupon not exposed to the decontaminant and that has no spores applied). Obvious visible damage might include structural damage, surface degradation, discoloration, or other aesthetic impacts.

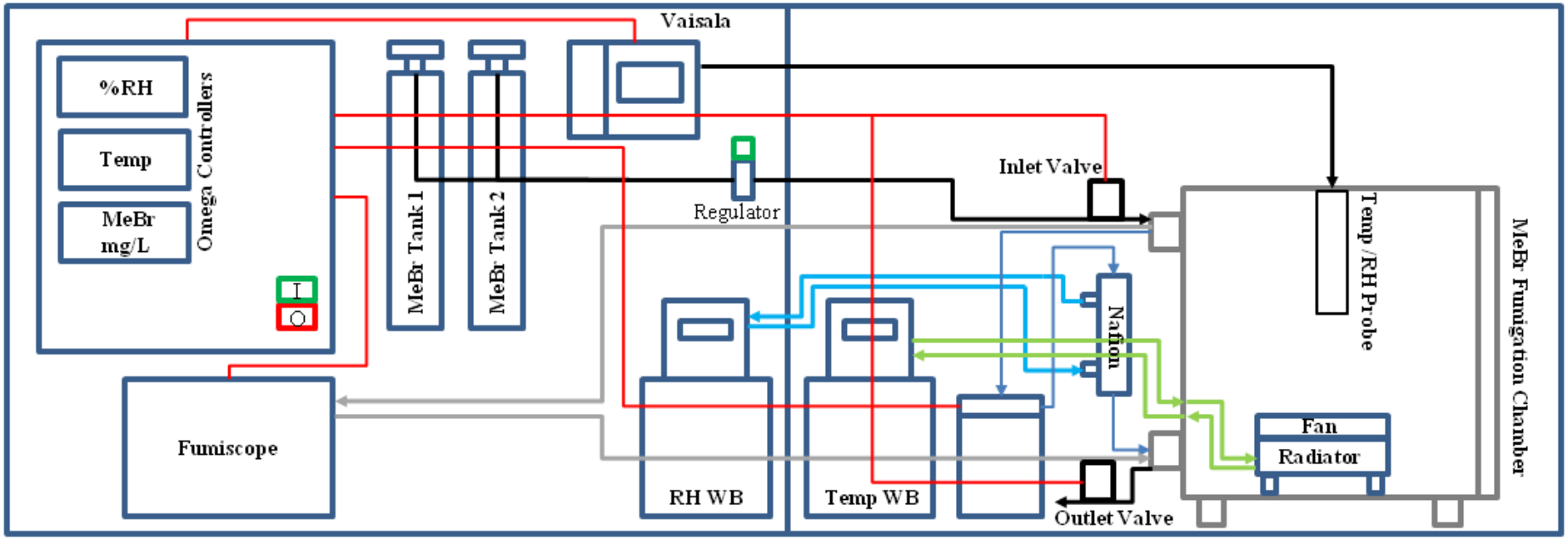
4.0 Fumigation Description and Procedures

Methyl bromide is a colorless and odorless volatile gas. Chloropicrin was added to the MeBr source gas (0.5 % chloropicrin, 99.5 % MeBr) as a warning irritant (lacrimator) for the safety of laboratory staff. The gas mixture was used at full strength and injected into the test chamber at the indicated target concentrations.

Figure 4-1 shows a schematic drawing of the MeBr test chamber and containment system. Decontamination testing was conducted inside an approximately 38 liter (L) stainless steel chamber. The chamber was insulated to prevent condensation on the inside chamber walls. As a means of secondary containment and laboratory personnel safety, this test chamber was housed inside a custom acrylic compact glove box (Plas Labs, Inc., Lansing, MI) that was hard-ducted to the facility exhaust system.

Temperature was controlled using a heated/cooled water bath, and relative humidity (RH) was elevated using a Nafion tube pervaporation system (controlled using a water bath). Temperature and RH in the test chamber was measured using an HMT368 temperature and humidity probe (Vaisala, Inc., Woburn, MA). Temperature, RH, and MeBr concentration were controlled with a CNI-822 controller (Omega Engineering, Stamford, CT) and were data-recorded every minute during the contact time using the associated iLOG software.

The MeBr concentration in the test chamber was measured continuously during the contact period using a Fumiscope™ Version 5.0 (Key Chemical and Equipment Company, Clearwater, FL). MeBr was added to the chamber, as necessary, to maintain the specified concentration within ±10 %. The Fumiscope meter was calibrated by the manufacturer for MeBr, displaying the concentration on a digital light-emitting diode (LED) display in ounces (oz) of MeBr per 1000 cubic feet (ft^3). One oz per 1000 ft^3 is approximately 257 parts per million (ppm) at 25 °C and is approximately 1 mg/L (independent of temperature). The Fumiscope meter included an air pump that pulled a gas sample from the test chamber through the thermal conductivity meter at a controlled rate and exhausted the gas back into the test chamber. Moisture was removed from the gas sample using a small paper filter before it was measured in the Fumiscope to eliminate interference from water.



WB = water bath
Indicates RH lines
Indicates Temperature Lines
Indicates MeBr loop from chamber to fumiscope
Indicates electrical lines to and from RH, temperature, and MeBr concentration controllers

Figure 4-1. Schematic of MeBr decontamination test chamber housed inside custom compact glove box.

A 9L Lock & Lock® airtight container (Lock & Lock, Farmers Branch, TX) served as the positive control chamber. Fixed humidity point salts[5] were added as a slurry to a separate container placed in the bottom of the MeBr positive control chamber. Sodium chloride was used to control the RH at 75 % and potassium carbonate to control the RH at 45 %. The control chamber was placed in an incubator (Thermo Scientific, Waltham, MA) for all tests and set to the appropriate temperature (i.e., 22, 27 or 32 °C). The temperature and RH of the positive control chamber were measured and datalogged using a HOBO® data logger model U12-11 (Onset Computer Corporation, Cape Cod, MA).

As in previous studies with MeBr[1], multiple coupons of each material were inoculated with the biological agent and placed on a wire rack inside the test chamber. Blank (i.e., uninoculated) and positive control (i.e., inoculated but not decontaminated) samples were also prepared for each material and were utilized with data from the test samples (inoculated and decontaminated) to determine decontamination efficacy.

The 20 MeBr tests were conducted at concentrations of either 212 or 300 mg/L, as shown in Table 2-1. Target contact times ranged from 18 to 72 hours, temperature from 22 to 32 °C and RH from 45 to 75 %. During each test run, inoculated test samples were placed inside the MeBr test chamber, and the chamber was sealed. The chamber was allowed sufficient time to equilibrate to the target temperature and RH prior to start of the run. Once the temperature and RH were stable, MeBr was slowly injected into the chamber until the target concentration was reached. The test chamber remained sealed until the end of the required contact time. At this time, the MeBr was turned off and the seal of the test chamber broken by removing the lid. The test chamber and BSC III were allowed to off-gas until the MeBr levels in the chamber reach 0 mg/L, which happened within minutes of lid removal. At this time, the samples were removed and processed as stated in Section 3.4.

5.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the *Quality Management Plan* (QMP) and the test/QA Plan. The QA/QC procedures and results are summarized below.

5.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets) and monitoring devices (e.g., thermometer, hygrometer) used at the time of evaluation were verified as being certified, calibrated, or validated.

5.2 QC Results

Quality control efforts conducted during decontaminant testing included positive control samples (inoculated, not decontaminated), procedural blanks (not inoculated, decontaminated), laboratory blank (not inoculated, not decontaminated), and inoculation control samples (analysis of the stock spore suspension).

All positive control results were within the target recovery range of 1 to 150 % of the inoculated spores, and all procedural and laboratory blanks met the criterion of no observed CFU for both organisms.

Inoculation control samples were taken from the spore suspension on the day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to inoculate the samples. The spore density levels met the QA target criterion of 1×10^9 CFU/mL (±1 log) for all tests.

5.2.1. Operational Parameters

The temperature, RH, and MeBr concentration during each test were controlled using Omega controllers, as described in Section 4.1. These controllers were set to the target conditions and allowed heat, RH, or MeBr to be injected as needed to stay within target ranges of ±2 °C, ±20 % RH and ±10 % MeBr. Readings were taken once every minute for the duration of the contact time. The actual operational parameters for each test are shown in Table 5-1 and reported as the average value ± SD.

Table 5-1. Actual Fumigation Conditions for MeBr Tests

Test Number	MeBr Concentration (mg/L)		Temperature (°C)		RH (%)		Contact Time (hours)†
	Target	Actual*	Target	Actual*	Target	Actual*	
1	212	213.39 ± 5.50	22	22.47 ± 0.31	45	45.03 ± 0.59	36
2	212	211.16 ± 2.95	22	22.20 ± 0.38	45	46.45 ± 1.87	48
3	212	211.74 ± 3.29	22	22.41 ± 0.16	75	75.29 ± 0.27	36
4	212	213.01 ± 4.23	27	27.58 ± 0.51	45	48.66 ± 8.90	36
5	212	212.38 ± 2.87	22	22.13 ± 0.13	75	74.90 ± 0.35	24
6	212	212.16 ± 2.96	27	27.14 ± 0.16	45	45.75 ± 1.63	48
7	212	212.17 ± 2.79	27	27.14 ± 0.23	75	75.98 ± 1.61	24
8	212	212.55 ± 3.14	27	27.13 ± 0.10	45	45.39 ± 1.52	48
9	212	211.97 ± 2.67	27	27.24 ± 0.14	75	75.72 ± 1.56	36
10	300	301.29 ± 3.07	22	22.32 ± 0.13	45	45.81 ± 1.30	48
11	212	210.81 ± 2.72	22	21.99 ± 0.19	45	46.07 ± 1.60	60
12	212	212.78 ± 3.21	32	32.14 ± 0.23	75	75.28 ± 1.64	24
13	212	212.16 ± 2.98	32	32.16 ± 0.16	45	45.99 ± 1.15	48
14	300	302.62 ± 3.49	22	22.20 ± 0.28	75	76.30 ± 2.36	24
15	300	302.87 ± 3.74	22	22.23 ± 0.11	45	46.10 ± 1.19	60
16	212	211.38 ± 2.93	32	32.17 ± 0.29	45	45.79 ± 0.84	60
17	300	300.87 ± 2.76	27	25.56 ± 0.58	75	81.16 ± 2.99	18
18	300	301.18 ± 2.86	27	27.28 ± 0.21	45	45.95 ± 1.03	60
19	212	212.10 ± 3.82	32	32.16 ± 0.28	45	46.23 ± 1.18	72
20	300	301.53 ± 4.41	32	32.14 ± 0.18	45	45.87 ± 0.89	60

* Data reported as average ± SD.

† Contact time did not deviate from target during any test.

5.3 Audits

5.3.1 Performance Evaluation Audit

Performance evaluation audits were conducted to assess the quality of the results obtained during these experiments. Table 5-2 summarizes the performance evaluation audits that were performed.

No performance evaluation audits were performed to confirm the concentration and purity of *B.a.* or surrogate spores because quantitative standards do not exist for these organisms. The control coupons and blanks support the spore measurements.

Table 5-2. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10 %	± 0.15 % to 2.5 %
Time	Compared to independent clock	± 2 seconds/hour	0 seconds/hour
Temperature	Compared to independent calibrated thermometer	± 2 °C	± 1.12 °C
Relative Humidity	Compare to independent calibrated hygrometer	± 20 %	± 1.52 %

5.3.2 Technical Systems Audit

Observations and findings from the technical systems audit (TSA) were documented and submitted to the laboratory staff lead for response. TSAs were conducted on August 9 and August 14, 2013, to ensure that the tests were being conducted in accordance with the appropriate test/QA plan and QMP. As part of the audit, test procedures were compared to those specified in the test/QA plan and data acquisition and handling procedures were reviewed. None of the findings of the TSA required corrective action.

5.3.3 Data Quality Audit

At least 10 % of the data acquired during the evaluation were audited. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

5.4 Test/Quality Assurance Plan Deviations

Section 3.2 of the test/QA plan states "The temperature and RH of the control and test chambers (excluding the MeBr test chamber) will be measured with a thermometer/hygrometer (Fisher Scientific Cat. No. S66283, Pittsburgh, PA), and the data will be recorded using a data logger (Onset Part No. U12-001, Bourne, MA)". For Test #9 started on 9/3/13, the HOBO® was inadvertently not launched inside the control chamber, resulting in no temperature or RH data at the end of the contact period. The parameters for Test #9 were as follows: 212 mg/L MeBr; 27 °C; 75 % RH; 36 hour contact time using *B.a.* Ames, Sterne and NNR1Δ1 against glass, ceiling tile, carpet and bare pine wood. The data for the decontamination samples were not affected.

5.5 QA/QC Reporting

Each assessment and audit was documented in accordance with the test/QA plan and QMP. For these tests, findings were noted (none significant) in the data quality audit, but no followup corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. Copies of the assessment reports were distributed to the EPA QA Manager and laboratory staff. QA/QC procedures were performed in accordance with the test/QA plan.

5.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports.

6.0 Summary of Results and Discussion

The decontamination efficacy of MeBr against virulent *B.a.* Ames and surrogates was evaluated at target concentrations of 212 and 300 mg/L, at a target temperatures of 22 to 32 °C, target RH of 45 and 75 %, and contact times ranging from 18 to 72 hours for a total of twenty tests. Table 6-1 shows the contact time required to achieve >6 LR (the level considered effective)[2] on all material types tested (glass, ceiling tile, carpet, painted wallboard paper, bare pine wood, and unpainted concrete) and at all target operational parameters. Actual operational parameters as measured were well within acceptable ranges and are detailed in Section 5. The detailed decontamination efficacy results are found in Appendix A. As seen in the table, a contact time of 36 hours was required to achieve > 6 LR of *B.a.* Ames when fumigating at 212 mg/L, 22 °C, and 75 % RH. Only 18 hours were required to achieve > 6 LR when the MeBr concentration was increased to 300 mg/L and temperature increased to 27 °C.

Table 6-1. Contact Time Required to Achieve >6 LR on all Materials*

Target MeBr Concentration (mg/L)	Target Temperature (° C)	Target RH (%)	Time (hours) Required to Achieve >6 LR on All Materials[c]				Test Number Reference[a]
			B.a. Ames	G.s.	*B.a.* NNR1Δ1	*B.a.* Sterne	
212	22	45	> 60	48	> 60	> 60	1, 2, 11
212	22	75	36	--[b]	> 36	--	3, 5
212	27	45	> 48	--	> 36	> 48	4, 6, 8
212	27	75	36	--	> 36	36	7, 9
212	32	45	> 72	--	> 72	> 72	13, 16, 19
212	32	75	24	--	> 24	24	12,
300	22	45	> 60	--	> 60	> 60	10, 15
300	22	75	24	--	> 24	> 24	14
300	27	45	> 60	--	> 60	> 60	18
300	27	75	18	--	> 18	> 18	17
300	32	45	> 60	--	> 60	> 60	20

* Materials tested were glass, ceiling tile, carpet, painted wallboard paper, bare pine wood and unpainted concrete.
[a] Detailed data from each test number can be referenced in Tables A-1 through A-4 in Appendix A.
[b] "--" Not tested.
[C] > dictates that >6 LR on all materials was not achieved at the contact time listed, and contact time was the longest tested.

6.1 Comparing Efficacy for the Different Species

A summary of the results comparing the average difference in decontamination efficacy for the microorganisms that were compared is shown in Table 6-2. Testing was first conducted using *G.s.* as a potential surrogate for *B.a.* Ames (Tests 1 & 2). The results showed that *G.s.* is less resistant than *B.a.* Ames to MeBr exposure. Therefore *G.s.* was eliminated from further testing, since a surrogate should be at least as resistant as the virulent strain. Thus additional potential surrogates, *B.a.* NNR1Δ1 and *B.a.* Sterne, were subsequently tested. In an attempt to evaluate all three organisms simultaneously, the number of materials tested was reduced due to the limited size of the MeBr test chamber. Painted wallboard paper and unpainted concrete were eliminated from testing after Test 8 as these materials were the easiest to decontaminate (highest LR values obtained). Refer to Appendix B for detailed efficacy results (e.g., results for each material) comparisons among microorganisms.

The results in Table 6-2 show that *B.a.* Sterne was more resistant (lower decontamination efficacy) to MeBr compared to *B.a.* Ames at the high RH condition (75 %), with the average

difference in efficacy ranging from -0.22 to -5.35 LR. In contrast, at 45 % RH, *B.a.* Sterne was always less resistant to MeBr than *B.a.* Ames.

The avirulent *B.a.* NNR1Δ1 was also tested alongside *B.a.* Ames in Tests 3-5 and 9-20. This organism was more resistant to MeBr than *B.a.* Ames in all tests performed, regardless of the temperature, RH, MeBr concentration, and contact time. However, the difference between *B.a.* Ames and the NNR1Δ1 strain was generally greater than the difference between the Ames and Sterne strains. In some tests (Tests 3 and 14), the difference in efficacy between *B.a.* Ames and the NNR1Δ1 strain was quite high (-6.33 and -6.18 LR, respectively).

Table 6-2. Summary of Average Differences in Efficacy between *B.a.* Ames and Avirulent Strains

Test Number	Target MeBr Concentration (mg/L)	Target Temperature (°C)	Target RH (%)	Contact Time (hour)	Average Difference in Efficacy		
					G.s.	*B.a.* NNR1Δ1	*B.a.* Sterne
1	212	22	45	36	2.26*	--[a]	--
2	212	22	45	48	1.10	--	--
11	212	22	45	60	--	-1.63	0.72
10	300	22	45	48	--	-1.55	1.86
15	300	22	45	60	--	-1.03	1.61
4	212	27	45	36	--	-1.29	--
6	212	27	45	48	--	--	1.74
8	212	27	45	48	--	--	1.54
18	300	27	45	60	--	-1.66	1.28
13	212	32	45	48	--	-1.59	1.31
16	212	32	45	60	--	-1.92	1.43
19	212	32	45	72	--	-2.06	1.63
20	300	32	45	60	--	-1.51	0.98
5	212	22	75	24	--	-2.20*	--
3	212	22	75	36	--	-6.33*	--
14	300	22	75	24	--	-6.16*	-5.35*
7	212	27	75	24	--	--	-3.75*
9	212	27	75	36	--	-1.83	-0.39
17	300	27	75	18	--	-4.96*	-3.67*
12	212	32	75	24	--	-2.54	-0.22

Results shown as average difference in efficacy (log reduction). A positive result indicates that the avirulent microorganism was inactivated to a higher degree (less resistant) than *B.a.* Ames. * An asterisk denotes a significant difference in efficacy.

[a] "--" Not tested at that condition.

6.2 Effects of Test Materials on MeBr efficacy for *B.a.* Ames

The LR results by material, for each test, are shown in the bar graphs in Figures 6-1 and 6-2. Differences in efficacy between two materials are significant if the 95 % CIs of the two efficacy results do not overlap. As discussed previously, testing was originally conducted (Tests 1 through 8) using six test materials. But in an attempt to evaluate three organisms at once (*B.a.* Ames, *B.a.* NNR1Δ1, and *B.a.* Sterne), the number of coupon materials tested was reduced due to the size of the MeBr test chamber. Painted wallboard and unpainted concrete were removed from testing in Tests 9 through 20, as these two material types generally exhibited higher efficacy than the other material types. Table 6-3 shows the average LR for each of the six materials in Tests 1-8, and the average LR for the four materials tested in Tests 9-20. In general, glass and wood were the materials most difficult to decontaminate (exhibited lower efficacy than

the other four material types). Further details on the decontamination efficacy results are found in Appendices A through C.

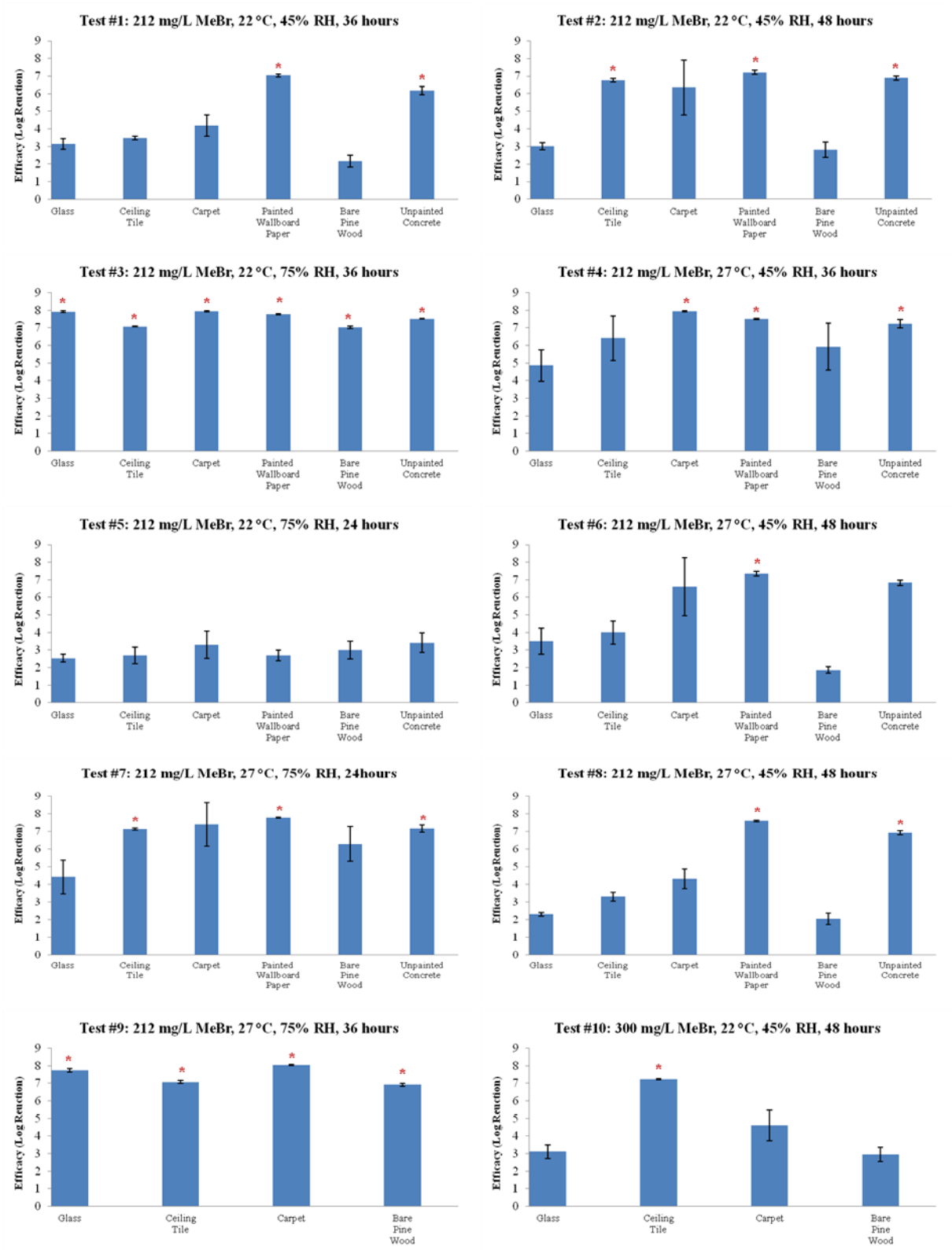


Figure 6-1. Summary of MeBr efficacy (Tests 1-10) results, by material, for *B. anthracis* Ames. Results shown are average log reduction ± CI.

*** Complete inactivation achieved**

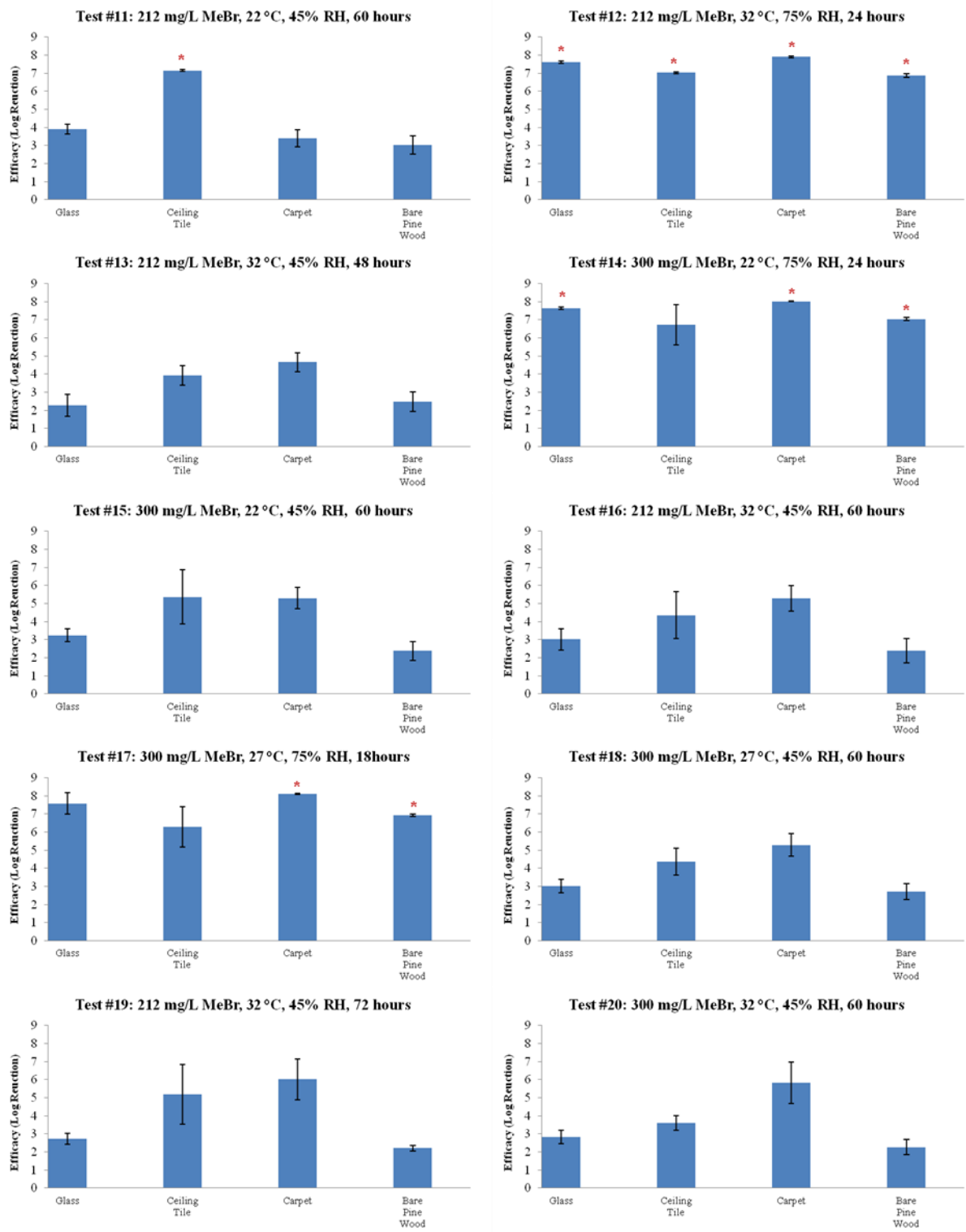


Figure 6-2. Summary of MeBr efficacy (Tests 11-20) results, by material, against *B. anthracis* Ames. Results shown in average log reduction ± CI.

*** Complete inactivation achieved**

Table 6-3. Summary of *B.a* Ames Average Log Reductions by Material Type

Material Type	Average (±SD) LR for Tests 1-8	Material Type	Average LR (±SD) for Tests 9-20
Glass	3.96 ± 1.83	Glass	4.56 ± 2.30
Ceiling Tile	5.11 ± 1.91	Ceiling Tile	5.69 ± 1.38
Carpet	6.00 ± 1.83	Carpet	6.04 ± 1.61
Painted Wallboard Paper	6.87 ± 1.71		
Bare Pine Wood	3.89 ± 2.15	Bare Pine Wood	4.02 ± 2.18
Unpainted Concrete	6.52 ± 1.32		

6.3 Effect of Temperature on Efficacy of MeBr against *B. anthracis* Ames

The decontamination efficacy of MeBr against virulent *B.a.* Ames was evaluated at target temperatures of 22, 27, or 32 °C. These temperatures were tested at various combinations of RH, MeBr concentration, and contact time; the results are organized by test condition in Figure 6-3 to visualize the effect of temperature. Additional analyses of the effect of temperature, including LR data for each specific material, are included in Tables C-1 and C-2 of Appendix C.

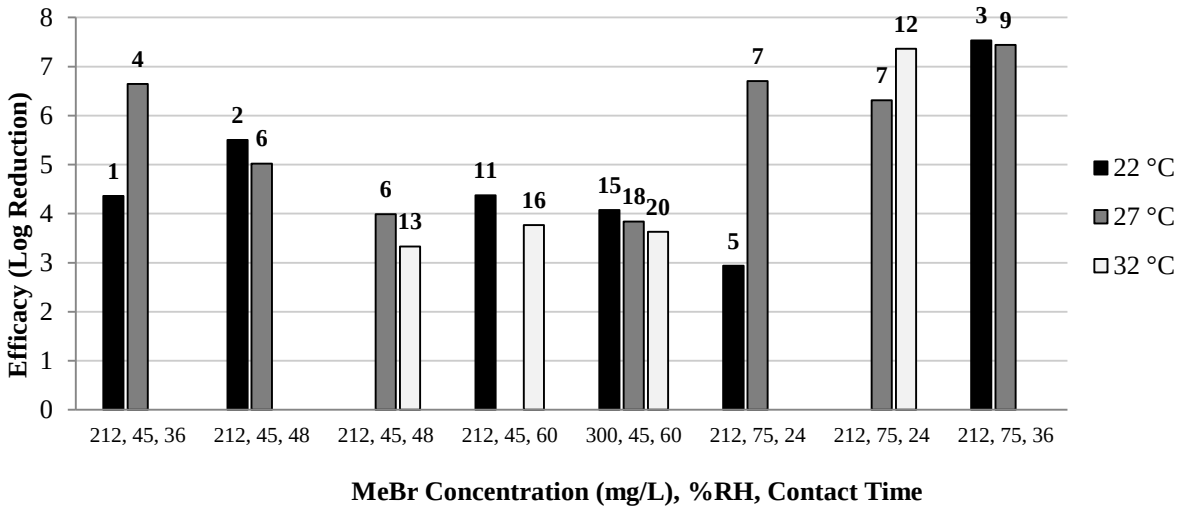


Figure 6-3. Effect of temperature on MeBr decontamination efficacy against *B. anthracis* Ames (Test numbers shown above each bar). Bars are the LR values averaged across the materials tested. In comparing with Test 13, which included only four materials, the second bar for Test 6 is the average of the same four materials. Similarly for the second bar shown for Test 7.

In general, increasing temperature (while holding all other test variables constant) either increased decontamination efficacy or had no significant effect on efficacy. At 45 % RH, there was only one test condition in which efficacy improved with increasing temperature; see comparison between Tests 1 and 4 in Figure 6-3. The remainder of the comparisons made for 45 % RH appear to show slightly reduced efficacy with increasing temperature, although these differences in efficacy are not statistically significant. At 75 % RH, there was just one test condition (out of three) in which increasing temperature resulted in a statistically significant

increase in efficacy; see comparison for Tests 5 and 7. The other two test conditions being compared did not show any significant increase in efficacy.

6.4 Effect of Relative Humidity on Efficacy of MeBr against *B. anthracis* Ames

The decontamination efficacy of MeBr against *B.a.* Ames was evaluated at target relative humidities of 45 or 75 %. The actual %RH conditions for each test are shown in Section 5. These RH levels were tested at various temperatures, MeBr concentrations, and contact times. The comparisons are shown in Figure 6-4 and detailed tabulated results to assess the effect of RH are summarized in Table C-3 of Appendix C.

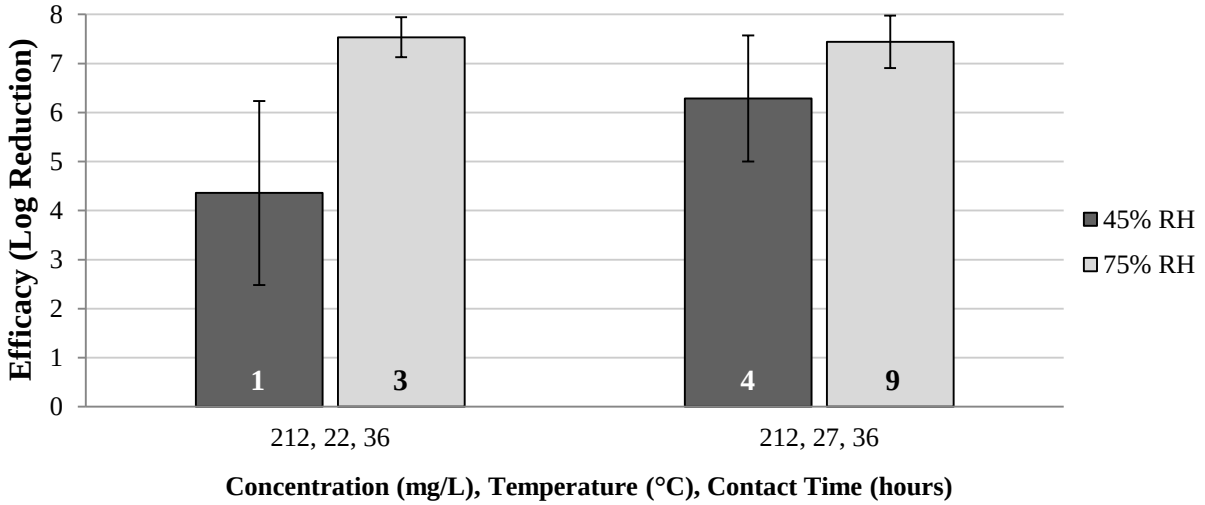


Figure 6-4. Effect of relative humidity on MeBr decontamination efficacy against *B. anthracis* Ames (Test numbers shown in each bar). Results shown as average log reduction ± standard deviation and corresponding test numbers are at the bottom of each bar.

The effect of increasing the %RH from 45 % to 75 % at low temperature (22 °C; compare Tests 1 and 3) and also at high temperature (27 °C; compare tests 4 and 9) was evaluated while keeping all other parameters constant (MeBr concentration and contact time). The average decontamination efficacy across all materials increased with increasing RH in both instances.

Overall, no test conducted at 45 % RH resulted in >6 LR of *B.a.* Ames for all materials tested.

6.5 Effect of MeBr Concentration on Efficacy against *B. anthracis* Ames

The decontamination efficacy of MeBr against virulent *B.a.* Ames was also evaluated at target concentrations of 212 and 300 mg/L. Refer to Section 5 for the actual MeBr concentrations achieved for each test. These concentrations were tested at various combinations of temperature and RH. Four test conditions showed results that could be compared to assess the effect of increasing MeBr concentration. These comparisons are shown in Figure 6-5, below, with detailed results for each material presented in Tables C-4 and C-5 of Appendix C.

For the three test conditions conducted at 45 % RH, there was no significant change in efficacy when increasing the MeBr concentration. Although only assessed once (between Tests 5 and 14), increasing the MeBr concentration from 212 to 300 mg/L in the presence of 75 % RH resulted in a significant increase in decontamination efficacy (4.47 LR), with complete inactivation or >6 LR on all materials tested.

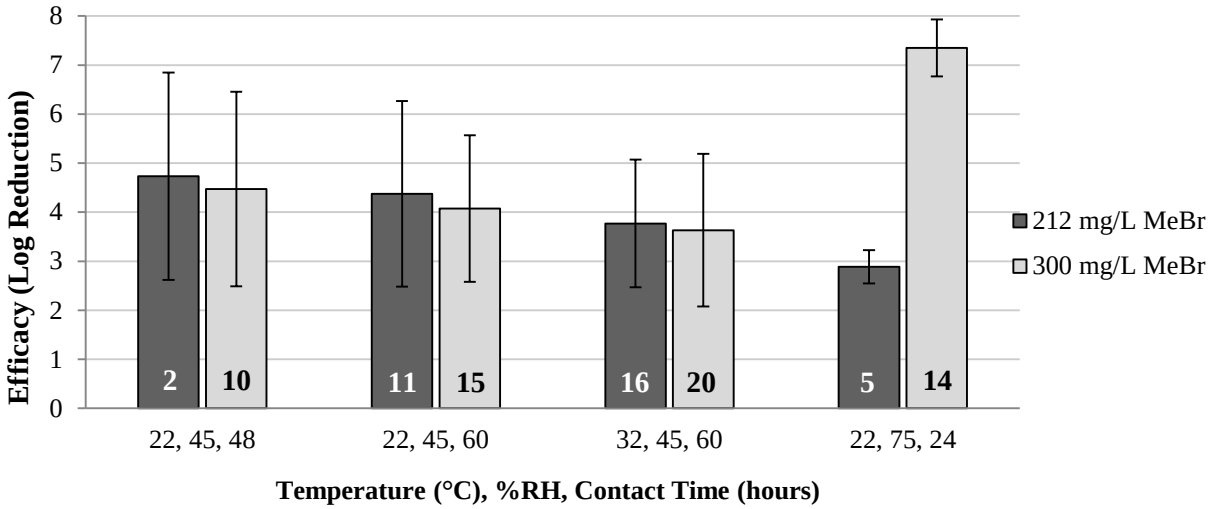


Figure 6-5. Summary of effect of increasing concentration on average MeBr decontamination efficacy for *B.a. Ames*. Results shown as average log reduction ± standard deviation and corresponding test numbers are at the bottom of each bar.

6.6 Effect of Contact Time on Efficacy of MeBr against *B. anthracis* Ames

The effect of increasing the contact time on the efficacy against *B.a.* Ames was also assessed. The contact times tested ranged from 18 to 72 hours; six sets of test conditions could be compared to assess the effect of increasing contact time. These comparisons are summarized in Figure 6-6 and presented in full detail in Tables C-6 and C-7 of Appendix C.

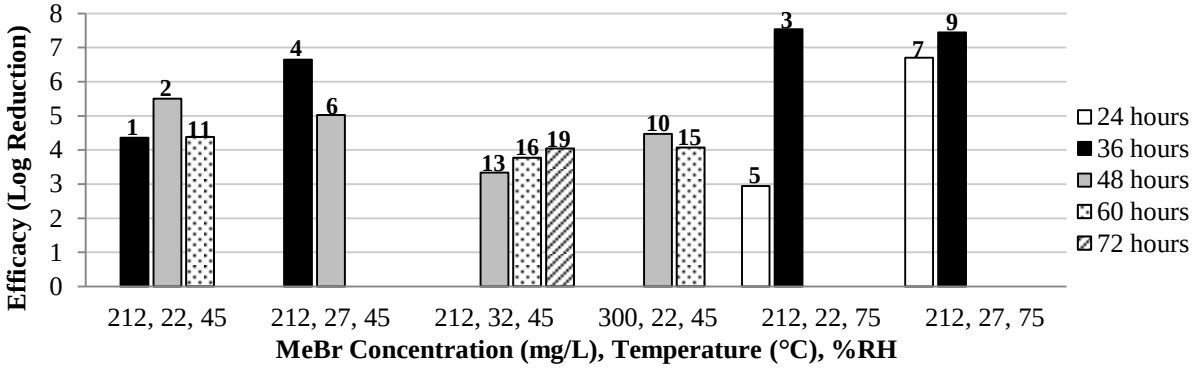


Figure 6-6. Summary of the effect of contact time on average MeBr decontamination efficacy against *B. anthracis Ames*. Corresponding test numbers are shown above each bar.

Similar to what we saw with the effect of increasing MeBr concentration (and to some extent, increasing temperature), there was no significant effect of increasing contact time on decontamination efficacy when fumigating at 45 % RH. At 75 % RH, there were two test conditions that could be compared to assess the effect of contact time. When increasing the contact time from 24 to 36 hours at 212 mg/L MeBr and 22 °C, there was a significant increase

in efficacy. These results show that when RH is low, RH is the predominant factor controlling efficacy.

6.7 Surface Damage to Materials

At the end of each decontamination test, the procedural blanks were visually compared to the laboratory blanks, and test coupons were visually compared to positive controls, to assess any impact MeBr may have had on each material type. Based on the visual appearance of the decontaminated coupons, there were no apparent changes in the color, reflectivity, or roughness of the six material surfaces after being exposed to MeBr.

6.8 Summary and Conclusion

This investigation focused on finding efficacious conditions when fumigating with MeBr at temperatures and RH levels lower than used in previous studies. Eliminating or reducing the need to humidify and/or heat a building would greatly facilitate MeBr fumigation when used to decontaminate a building contaminated with *B. anthracis* spores. Another objective of the study was to compare the decontamination results for *B. anthracis* (Ames) with avirulent spore-forming microorganisms, to assess their potential as surrogates for use in future studies with MeBr.

This study shows the important role that RH plays when fumigating with MeBr. There were no tests in which >6 LR of *B.a.* Ames was achieved on all materials when fumigating at 45 % RH. When fumigating at 45 % RH, increasing the MeBr concentration, temperature, or contact time generally did not improve decontamination efficacy. In contrast, when fumigating at 75 % RH, increasing the MeBr concentration, temperature and contact time did generally improve efficacy. For example, a contact time of 36 hours was required to achieve > 6 LR of *B.a.* Ames on all materials when fumigating at 212 mg/L, 22 °C, and 75 % RH. However, only 18 hours of contact time were required to achieve > 6 LR of *B.a.* Ames on all materials when the MeBr concentration was increased to 300 mg/L and temperature increased to 27 °C.

From two of the initial tests, the study showed that *G.s.* spores are less resistant to MeBr than *B.a.* Ames. Therefore *G.s.* was eliminated from further testing, since a surrogate should be at least as resistant as the virulent strain. In every test conducted with the NNR1Δ1 strain, the NNR1Δ1 strain was always more resistant than the Ames strain, and in a few tests, the average difference in LR with the Ames strain was more than 6.0. In the tests with the Sterne strain, Sterne was always inactivated to a higher degree than the Ames strain when fumigating at 45 % RH. But when fumigating at 75 % RH, the Sterne strain was more resistant than *B.a.* Ames.

Impact of Study

This work provides information on the efficacy of MeBr fumigation to decontaminate materials that have been contaminated with *B. anthracis* spores. Such results may be useful in the development of guidance to aid in deployment of MeBr fumigation after a wide-area release of *B. anthracis* spores. In particular, these results will provide decision makers with information for effectively using MeBr at temperatures and RH levels lower than has been recommended previously, which will facilitate its use. This report also provides data to assist in selection of an avirulent surrogate for *B. anthracis* Ames, for use in future field studies and additional lab-based investigations utilizing MeBr.

7.0 References

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Appendix A
Detailed Test Results

Efficacy Results

The detailed decontamination efficacy results for methyl bromide against *B.a.* Ames, *G.s.*, *B.a.* NNR1Δ1, and *B.a.* Sterne on six material types (glass, ceiling tile, carpet, painted wallboard paper, bare pine wood and unpainted concrete) are shown in Tables A-1 through A-4. Zero CFU were observed on all laboratory and procedural blanks.

Table A-1. Inactivation of *B. anthracis* Ames Spores using Methyl Bromide[a]

Test Number	Target Parameters		Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* Ames (CFU/coupon)		Decontamination Efficacy ± CI[d]
	Concentration (mg/L) / Contact Time (hr)	Temp (°C) / RH (%)			Positive Control[b]	Test Coupon[c]	
1	212/36	22/45	Glass	5.97×10^7	$3.13 \pm 0.69 \times 10^7$	$2.84 \pm 2.24 \times 10^4$	3.14 ± 0.30
			Ceiling Tile		$7.67 \pm 1.66 \times 10^6$	$2.55 \pm 0.39 \times 10^3$	3.47 ± 0.11
			Carpet		$4.30 \pm 0.94 \times 10^7$	$0.70 \pm 1.10 \times 10^4$	4.18 ± 0.60
			Painted Wallboard Paper		$1.11 \pm 0.24 \times 10^7$	0.00 ± 0.00	≥7.04 ± 0.08
			Bare Pine Wood		$3.23 \pm 0.44 \times 10^6$	$2.99 \pm 2.39 \times 10^4$	2.16 ± 0.34
			Unpainted Concrete		$1.76 \pm 1.11 \times 10^6$	0.00 ± 0.00	≥6.17 ± 0.25
2	212/48	22/45	Glass	7.00×10^7	$3.67 \pm 0.30 \times 10^7$	$4.01 \pm 1.76 \times 10^4$	3.00 ± 0.20
			Ceiling Tile		$5.90 \pm 1.34 \times 10^6$	0.00 ± 0.00	≥6.76 ± 0.09
			Carpet		$4.45 \pm 0.56 \times 10^7$	$0.71 \pm 1.03 \times 10^3$	6.35 ± 1.55
			Painted Wallboard Paper		$1.72 \pm 0.63 \times 10^7$	0.00 ± 0.00	≥7.21 ± 0.12
			Bare Pine Wood		$5.70 \pm 1.59 \times 10^6$	$1.20 \pm 0.83 \times 10^4$	2.82 ± 0.44
			Unpainted Concrete		$8.03 \pm 2.34 \times 10^6$	0.00 ± 0.00	≥6.89± 0.11
3	212/36	22/75	Glass	1.07×10^8	$8.36 \pm 0.78 \times 10^7$	0.00 ± 0.00	≥7.92 ± 0.04
			Ceiling Tile		$1.18 \pm 0.05 \times 10^7$	0.00 ± 0.00	≥7.07 ± 0.02
			Carpet		$8.55 \pm 0.79 \times 10^7$	0.00 ± 0.00	≥7.93 ± 0.04
			Painted Wallboard Paper		$5.84 \pm 0.59 \times 10^7$	0.00 ± 0.00	≥7.76 ± 0.04
			Bare Pine Wood		$1.07 \pm 0.18 \times 10^7$	0.00 ± 0.00	≥7.02 ± 0.06
			Unpainted Concrete		$3.45 \pm 1.19 \times 10^6$	0.00 ± 0.00	≥7.51± 0.15
4	212/36	27/45	Glass	8.80×10^7	$6.57 \pm 1.64 \times 10^7$	$3.07 \pm 3.21 \times 10^3$	4.86 ± 0.90
			Ceiling Tile		$1.13 \pm 0.15 \times 10^7$	$3.35 \pm 7.46 \times 10^2$	6.41 ± 1.26
			Carpet		$8.98 \pm 0.61 \times 10^7$	0.00 ± 0.00	≥7.95 ± 0.03
			Painted Wallboard Paper		$3.18 \pm 0.43 \times 10^7$	0.00 ± 0.00	≥7.50 ± 0.05
			Bare Pine Wood		$9.77 \pm 3.27 \times 10^6$	$0.48 \pm 1.02 \times 10^3$	5.93 ± 1.34
			Unpainted Concrete		$2.01 \pm 1.31 \times 10^7$	0.00 ± 0.00	≥7.23± 0.24
5	212/24	22/75	Glass	1.02×10^8	$5.03 \pm 1.01 \times 10^7$	$1.60 \pm 0.91 \times 10^5$	2.54 ± 0.22
			Ceiling Tile		$1.10 \pm 0.12 \times 10^7$	$3.69 \pm 3.56 \times 10^4$	2.70 ± 0.48
			Carpet		$7.51 \pm 1.33 \times 10^7$	$9.85 \pm 8.62 \times 10^5$	3.31 ± 0.78
			Painted Wallboard Paper		$4.38 \pm 0.66 \times 10^7$	$1.08 \pm 0.62 \times 10^5$	2.69 ± 0.30
			Bare Pine Wood		$5.63 \pm 1.40 \times 10^6$	$1.35 \pm 2.30 \times 10^4$	2.99 ± 0.50
			Unpainted Concrete		$2.10 \pm 0.93 \times 10^7$	$1.65 \pm 2.40 \times 10^4$	3.41± 0.56
6	212/48	27/45	Glass	1.19×10^8	$6.28 \pm 1.24 \times 10^7$	$6.20 \pm 7.55 \times 10^4$	3.49 ± 0.74
			Ceiling Tile		$1.09 \pm 0.10 \times 10^7$	$2.41 \pm 2.31 \times 10^3$	4.00 ± 0.65
			Carpet		$9.08 \pm 0.49 \times 10^7$	$1.59 \pm 3.09 \times 10^3$	6.60 ± 1.66
			Painted Wallboard Paper		$2.31 \pm 0.79 \times 10^7$	0.00 ± 0.00	≥7.34 ± 0.14
			Bare Pine Wood		$6.15 \pm 0.82 \times 10^6$	$9.06 \pm 3.64 \times 10^4$	1.86 ± 0.19
			Unpainted Concrete		$7.39 \pm 3.26 \times 10^6$	0.00 ± 0.00	≥6.84± 0.15
7	212/24	27/75	Glass	1.04×10^8	$6.15 \pm 0.95 \times 10^7$	$6.97 \pm 6.21 \times 10^3$	4.42 ± 0.96
			Ceiling Tile		$1.40 \pm 0.20 \times 10^7$	0.00 ± 0.00	≥7.14 ± 0.06
			Carpet		$1.09 \pm 0.13 \times 10^8$	$2.95 \pm 6.57 \times 10^2$	7.40 ± 1.24
			Painted Wallboard Paper		$6.15 \pm 0.39 \times 10^7$	0.00 ± 0.00	≥7.79 ± 0.02
			Bare Pine Wood		$6.78 \pm 3.74 \times 10^6$	$0.61 \pm 1.34 \times 10^2$	6.29 ± 0.99
			Unpainted Concrete		$1.65 \pm 0.95 \times 10^7$	0.00 ± 0.00	≥7.17± 0.20
8	212/48	27/45	Glass	1.35×10^8	$6.91 \pm 1.22 \times 10^7$	$3.54 \pm 0.67 \times 10^5$	2.29 ± 0.10
			Ceiling Tile		$1.33 \pm 0.26 \times 10^7$	$7.78 \pm 4.57 \times 10^3$	3.29 ± 0.26
			Carpet		$1.26 \pm 0.25 \times 10^8$	$9.61 \pm 6.63 \times 10^3$	4.31 ± 0.55
			Painted Wallboard Paper		$4.03 \pm 0.52 \times 10^7$	0.00 ± 0.00	≥7.60 ± 0.05
			Bare Pine Wood		$8.76 \pm 4.18 \times 10^6$	$8.11 \pm 5.02 \times 10^4$	2.03 ± 0.32
			Unpainted Concrete		$9.16 \pm 3.03 \times 10^6$	0.00 ± 0.00	≥6.94± 0.12

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated. [d] CI = confidence interval (± 1.96 × SE).

Table A-1. Inactivation of *B. anthracis* Ames Spores using Methyl Bromide[a] *(Continued)*

Test Number	Target Parameters: Concentration (mg/L) / Contact Time (hr)	Target Parameters: Temp (°C) / RH (%)	Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* Ames (CFU/coupon): Positive Control[b]	Mean Recovered *B.a.* Ames (CFU/coupon): Test Coupon[c]	Decontamination Efficacy ± CI[d]
9	212/36	27/75	Glass	1.45×10^8	$5.65 \pm 1.58 \times 10^7$	0.00 ± 0.00	$\geq 7.74 \pm 0.10$
			Ceiling Tile		$1.20 \pm 0.26 \times 10^7$	0.00 ± 0.00	$\geq 7.07 \pm 0.08$
			Carpet		$1.11 \pm 0.09 \times 10^8$	0.00 ± 0.00	$\geq 8.04 \pm 0.03$
			Bare Pine Wood		$8.53 \pm 1.69 \times 10^6$	0.00 ± 0.00	$\geq 6.92 \pm 0.08$
10	300/48	22/45	Glass	1.15×10^8	$3.96 \pm 0.61 \times 10^7$	$4.62 \pm 4.96 \times 10^4$	3.11 ± 0.38
			Ceiling Tile		$1.71 \pm 0.19 \times 10^7$	0.00 ± 0.00	$\geq 7.23 \pm 0.04$
			Carpet		$9.23 \pm 1.42 \times 10^7$	$6.95 \pm 6.08 \times 10^3$	4.60 ± 0.89
			Bare Pine Wood		$9.67 \pm 5.48 \times 10^6$	$1.41 \pm 1.29 \times 10^4$	2.95 ± 0.42
11	212/60	22/45	Glass	9.67×10^7	$5.61 \pm 1.42 \times 10^7$	$8.15 \pm 5.43 \times 10^3$	3.90 ± 0.27
			Ceiling Tile		$1.47 \pm 0.24 \times 10^7$	0.00 ± 0.00	$\geq 7.16 \pm 0.06$
			Carpet		$8.98 \pm 2.83 \times 10^7$	$5.26 \pm 4.77 \times 10^4$	3.41 ± 0.47
			Bare Pine Wood		$8.13 \pm 2.76 \times 10^6$	$1.52 \pm 2.23 \times 10^4$	3.03 ± 0.52
12	212/24	32/75	Glass	1.12×10^8	$4.08 \pm 0.77 \times 10^7$	0.00 ± 0.00	$\geq 7.61 \pm 0.07$
			Ceiling Tile		$1.10 \pm 0.41 \times 10^7$	0.00 ± 0.00	$\geq 7.04 \pm 0.05$
			Carpet		$8.13 \pm 1.20 \times 10^7$	0.00 ± 0.00	$\geq 7.91 \pm 0.06$
			Bare Pine Wood		$8.01 \pm 2.53 \times 10^6$	0.00 ± 0.00	$\geq 6.89 \pm 0.10$
13	212/48	32/45	Glass	1.14×10^8	$5.31 \pm 1.03 \times 10^7$	$5.57 \pm 4.95 \times 10^5$	2.28 ± 0.61
			Ceiling Tile		$1.29 \pm 0.31 \times 10^7$	$2.36 \pm 1.71 \times 10^3$	3.93 ± 0.54
			Carpet		$1.07 \pm 0.06 \times 10^8$	$4.01 \pm 3.11 \times 10^3$	4.65 ± 0.52
			Bare Pine Wood		$1.05 \pm 0.50 \times 10^6$	$5.31 \pm 4.45 \times 10^4$	2.47 ± 0.53
14	300/24	22/75	Glass	1.31×10^8	$4.22 \pm 0.71 \times 10^7$	0.00 ± 0.00	$\geq 7.62 \pm 0.07$
			Ceiling Tile		$2.02 \pm 0.84 \times 10^7$	$1.34 \pm 2.98 \times 10^2$	6.72 ± 1.12
			Carpet		$1.05 \pm 0.07 \times 10^8$	0.00 ± 0.00	$\geq 8.02 \pm 0.03$
			Bare Pine Wood		$1.11 \pm 0.28 \times 10^7$	0.00 ± 0.00	$\geq 7.04 \pm 0.09$
15	300/60	22/45	Glass	1.27×10^8	$4.63 \pm 1.16 \times 10^7$	$3.41 \pm 2.82 \times 10^4$	3.25 ± 0.37
			Ceiling Tile		$1.46 \pm 0.24 \times 10^7$	$1.06 \pm 1.55 \times 10^3$	5.36 ± 1.50
			Carpet		$7.98 \pm 2.60 \times 10^7$	$7.14 \pm 6.82 \times 10^2$	5.30 ± 0.59
			Bare Pine Wood		$8.61 \pm 1.76 \times 10^6$	$5.76 \pm 4.40 \times 10^4$	2.38 ± 0.53
16	212/60	32/45	Glass	9.23×10^7	$7.23 \pm 2.32 \times 10^7$	$1.27 \pm 1.45 \times 10^5$	3.03 ± 0.59
			Ceiling Tile		$1.09 \pm 0.16 \times 10^7$	$1.78 \pm 1.02 \times 10^3$	4.36 ± 1.31
			Carpet		$1.04 \pm 0.11 \times 10^8$	$1.31 \pm 1.39 \times 10^3$	5.29 ± 0.71
			Bare Pine Wood		$6.32 \pm 0.60 \times 10^6$	$6.23 \pm 7.31 \times 10^4$	2.40 ± 0.68
17	300/18	27/75	Glass	1.08×10^8	$7.77 \pm 0.80 \times 10^7$	$0.75 \pm 1.44 \times 10^1$	7.58 ± 0.60
			Ceiling Tile		$1.46 \pm 0.37 \times 10^7$	$1.34 \pm 2.79 \times 10^2$	6.29 ± 1.12
			Carpet		$1.30 \pm 0.13 \times 10^8$	0.00 ± 0.00	$\geq 8.11 \pm 0.04$
			Bare Pine Wood		$8.42 \pm 1.30 \times 10^6$	0.00 ± 0.00	$\geq 6.92 \pm 0.06$
18	300/60	27/45	Glass	1.13×10^8	$7.78 \pm 1.42 \times 10^7$	$1.02 \pm 0.78 \times 10^5$	3.01 ± 0.38
			Ceiling Tile		$1.90 \pm 0.98 \times 10^7$	$1.77 \pm 1.81 \times 10^3$	4.36 ± 0.75
			Carpet		$1.39 \pm 0.23 \times 10^8$	$2.11 \pm 3.60 \times 10^3$	5.28 ± 0.62
			Bare Pine Wood		$1.59 \pm 1.04 \times 10^7$	$3.68 \pm 2.86 \times 10^4$	2.71 ± 0.44
19	212/72	32/45	Glass	1.21×10^8	$7.10 \pm 2.40 \times 10^7$	$1.62 \pm 1.35 \times 10^5$	2.72 ± 0.31
			Ceiling Tile		$1.58 \pm 0.24 \times 10^7$	$2.37 \pm 3.57 \times 10^3$	5.20 ± 1.65
			Carpet		$1.15 \pm 0.23 \times 0^8$	$4.73 \pm 4.26 \times 10^2$	6.02 ± 1.13
			Bare Pine Wood		$8.41 \pm 1.02 \times 10^6$	$5.59 \pm 2.60 \times 10^4$	2.21 ± 0.16
20	300/60	32/45	Glass	1.27×10^8	$4.27 \pm 0.79 \times 10^7$	$7.98 \pm 4.70 \times 10^4$	2.84 ± 0.37
			Ceiling Tile		$1.22 \pm 0.29 \times 10^7$	$4.52 \pm 4.08 \times 10^3$	3.60 ± 0.41
			Carpet		$9.33 \pm 0.37 \times 10^7$	$7.07 \pm 8.41 \times 10^2$	5.82 ± 1.15
			Bare Pine Wood		$8.39 \pm 1.25 \times 10^6$	$0.79 \pm 1.08 \times 10^5$	2.27 ± 0.42

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).

Table A-2. Inactivation of *G. stearothermophilus* Spores using Methyl Bromide[a]

Test Number	Target Parameters: Concentration (mg/L) / Contact Time (hr)	Target Parameters: Temp (°C) / RH (%)	Material	Inoculum (CFU/coupon)	Mean Recovered G.s. (CFU/coupon): Positive Control[b]	Mean Recovered G.s. (CFU/coupon): Test Coupon[c]	Decontamination Efficacy ± CI[d]
1	212/36	22/45	Glass	6.80×10^7	$5.37 \pm 0.91 \times 10^7$	0.00 ± 0.00	≥7.72 ± 0.07
			Ceiling Tile		$4.22 \pm 0.94 \times 10^5$	$3.39 \pm 5.72 \times 10^1$	4.89 ± 0.90
			Carpet		$1.60 \pm 1.10 \times 10^7$	$1.41 \pm 2.94 \times 10^1$	6.77 ± 0.75
			Painted Wallboard Paper		$6.69 \pm 0.43 \times 10^7$	0.00 ± 0.00	≥7.82 ± 0.02
			Bare Pine Wood		$7.87 \pm 6.67 \times 10^5$	0.00 ± 0.00	≥5.75 ± 0.36
			Unpainted Concrete		$1.03 \pm 0.64 \times 10^7$	0.00 ± 0.00	≥6.78 ± 0.62
2	212/48	22/45	Glass	7.77×10^7	$5.81 \pm 0.94 \times 10^7$	0.00 ± 0.00	≥7.76 ± 0.06
			Ceiling Tile		$1.32 \pm 1.37 \times 10^5$	0.00 ± 0.00	≥5.00 ± 0.29
			Carpet		$1.65 \pm 0.66 \times 10^7$	0.00 ± 0.00	≥7.19 ± 0.14
			Painted Wallboard Paper		$2.89 \pm 1.30 \times 10^7$	0.00 ± 0.00	≥7.43 ± 0.17
			Bare Pine Wood		$1.59 \pm 3.34 \times 10^6$	0.00 ± 0.00	≥5.37 ± 0.75
			Unpainted Concrete		$7.53 \pm 3.50 \times 10^6$	0.00 ± 0.00	≥6.85± 0.15

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).

Table A-3. Inactivation of *B. anthracis* NNR1Δ1 Spores using Methyl Bromide[a]

Test Number	Target Parameters: Concentration (mg/L) / Contact Time (hr)	Target Parameters: Temp (°C) / RH (%)	Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* NNR1Δ1 (CFU/coupon): Positive Control[b]	Mean Recovered *B.a.* NNR1Δ1 (CFU/coupon): Test Coupon[c]	Decontamination Efficacy ± CI[d]
3	212/36	22/75	Glass	9.33×10^7	$2.21 \pm 1.09 \times 10^6$	$5.35 \pm 1.47 \times 10^5$	0.60 ± 0.20
			Ceiling Tile		$1.28 \pm 0.41 \times 10^6$	$1.19 \pm 1.12 \times 10^5$	1.12 ± 0.31
			Carpet		$4.83 \pm 0.86 \times 10^7$	$1.38 \pm 0.44 \times 10^6$	1.56 ± 0.14
			Painted Wallboard Paper		$1.40 \pm 0.42 \times 10^7$	$1.81 \pm 0.30 \times 10^6$	0.88 ± 0.15
			Bare Pine Wood		$1.28 \pm 0.45 \times 10^7$	$2.15 \pm 1.32 \times 10^5$	1.80 ± 0.24
			Unpainted Concrete		$2.00 \pm 1.10 \times 10^7$	$1.17 \pm 0.64 \times 10^6$	1.26 ± 0.34
4	212/36	27/45	Glass	1.01×10^8	$8.05 \pm 1.36 \times 10^7$	$9.49 \pm 5.72 \times 10^5$	1.97 ± 0.21
			Ceiling Tile		$4.30 \pm 1.43 \times 10^6$	$1.74 \pm 2.55 \times 10^2$	5.57 ± 1.26
			Carpet		$8.49 \pm 0.81 \times 10^7$	$1.85 \pm 1.83 \times 10^3$	4.89 ± 0.51
			Painted Wallboard Paper		$5.99 \pm 1.26 \times 10^7$	0.00 ± 0.00	≥7.77 ± 0.08
			Bare Pine Wood		$2.43 \pm 1.32 \times 10^7$	$8.26 \pm 9.71 \times 10^2$	5.08 ± 1.19
			Unpainted Concrete		$8.13 \pm 3.32 \times 10^6$	0.00 ± 0.00	≥6.88 ± 0.18
5	212/24	22/75	Glass	1.12×10^8	$8.24 \pm 0.71 \times 10^7$	$3.72 \pm 0.50 \times 10^7$	0.35 ± 0.06
			Ceiling Tile		$6.52 \pm 2.04 \times 10^6$	$1.07 \pm 0.40 \times 10^6$	0.80 ± 0.21
			Carpet		$7.42 \pm 1.38 \times 10^7$	$1.57 \pm 0.34 \times 10^7$	0.68 ± 0.12
			Painted Wallboard Paper		$5.85 \pm 0.20 \times 10^7$	$1.44 \pm 0.16 \times 10^7$	0.61 ± 0.04
			Bare Pine Wood		$7.51 \pm 1.80 \times 10^6$	$1.12 \pm 0.43 \times 10^6$	0.84 ± 0.19
			Unpainted Concrete		$1.20 \pm 0.38 \times 10^7$	$1.20 \pm 0.88 \times 10^6$	1.16 ± 0.47
9	212/36	27/75	Glass	8.13×10^7	$6.00 \pm 2.85 \times 10^7$	$2.20 \pm 2.87 \times 10^5$	2.79 ± 0.68
			Ceiling Tile		$3.89 \pm 3.07 \times 10^6$	0.00 ± 0.00	≥6.48 ± 0.30
			Carpet		$8.29 \pm 1.81 \times 10^7$	0.00 ± 0.00	≥7.91 ± 0.08
			Bare Pine Wood		$3.83 \pm 2.63 \times 10^6$	$5.20 \pm 7.73 \times 10^2$	5.26 ± 1.51
10	300/48	22/45	Glass	1.68×10^8	$5.82 \pm 0.69 \times 10^7$	$1.39 \pm 0.10 \times 10^7$	0.62 ± 0.06
			Ceiling Tile		$1.01 \pm 0.20 \times 10^7$	$0.73 \pm 1.62 \times 10^2$	6.29 ± 1.40
			Carpet		$8.82 \pm 0.58 \times 10^7$	$2.26 \pm 1.31 \times 10^6$	1.65 ± 0.24
			Bare Pine Wood		$7.30 \pm 0.76 \times 10^6$	$9.64 \pm 8.96 \times 10^3$	3.13 ± 0.52
11	212/60	22/45	Glass	1.31×10^8	$8.28 \pm 1.30 \times 10^7$	$1.14 \pm 0.26 \times 10^7$	0.87 ± 0.11
			Ceiling Tile		$5.30 \pm 1.18 \times 10^6$	$1.60 \pm 1.96 \times 10^2$	5.33 ± 1.14
			Carpet		$8.68 \pm 1.29 \times 10^7$	$4.45 \pm 1.29 \times 10^6$	1.30 ± 0.13
			Bare Pine Wood		$7.75 \pm 2.96 \times 10^6$	$3.59 \pm 3.17 \times 10^3$	3.48 ± 0.48
12	212/24	32/75	Glass	1.27×10^8	$4.80 \pm 1.14 \times 10^7$	$9.24 \pm 3.46 \times 10^5$	1.73 ± 0.19
			Ceiling Tile		$4.47 \pm 0.82 \times 10^6$	0.00 ± 0.00	≥6.64 ± 0.07
			Carpet		$5.23 \pm 1.25 \times 10^7$	$4.08 \pm 8.90 \times 10^1$	7.25 ± 0.91
			Bare Pine Wood		$5.17 \pm 1.85 \times 10^6$	$1.57 \pm 1.93 \times 10^3$	3.69 ± 0.40
13	212/48	32/45	Glass	1.02×10^8	$8.09 \pm 0.71 \times 10^7$	$2.08 \pm 0.65 \times 10^7$	0.60 ± 0.11
			Ceiling Tile		$1.22 \pm 0.45 \times 10^6$	$3.15 \pm 2.50 \times 10^3$	2.67 ± 0.36
			Carpet		$7.98 \pm 1.20 \times 10^7$	$1.95 \pm 0.59 \times 10^6$	1.63 ± 0.16
			Bare Pine Wood		$1.32 \pm 0.95 \times 10^6$	$9.90 \pm 3.50 \times 10^3$	2.06 ± 0.29

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).

Table A-3. Inactivation of *B. anthracis* NNR1Δ1 Spores using Methyl Bromide[a] *(Continued)*

Test Number	Target Parameters: Concentration (mg/L) / Contact Time (hr)	Target Parameters: Temp (°C) / RH (%)	Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* NNR1Δ1 (CFU/coupon): Positive Control[b]	Mean Recovered *B.a.* NNR1Δ1 (CFU/coupon): Test Coupon[c]	Decontamination Efficacy ± CI[d]
14	300/24	22/75	Glass	1.22×10^8	$4.38 \pm 0.89 \times 10^7$	$4.15 \pm 1.04 \times 10^6$	1.03 ± 0.13
			Ceiling Tile		$3.72 \pm 0.43 \times 10^6$	$1.89 \pm 1.15 \times 10^5$	1.36 ± 0.24
			Carpet		$4.62 \pm 1.55 \times 10^7$	$8.12 \pm 2.29 \times 10^6$	0.75 ± 0.17
			Bare Pine Wood		$1.08 \pm 0.50 \times 10^7$	$2.77 \pm 1.09 \times 10^5$	1.63 ± 0.28
15	300/60	22/45	Glass	1.12×10^8	$7.33 \pm 2.44 \times 10^7$	$9.57 \pm 2.43 \times 10^6$	0.87 ± 0.19
			Ceiling Tile		$4.31 \pm 1.19 \times 10^6$	$4.07 \pm 7.20 \times 10^1$	5.87 ± 0.93
			Carpet		$6.98 \pm 1.63 \times 10^7$	$1.21 \pm 0.74 \times 10^6$	1.85 ± 0.33
			Bare Pine Wood		$4.08 \pm 2.02 \times 10^6$	$1.01 \pm 0.55 \times 10^3$	3.60 ± 0.33
16	212/60	32/45	Glass	1.23×10^8	$4.68 \pm 1.58 \times 10^7$	$1.64 \pm 0.86 \times 10^7$	0.48 ± 0.22
			Ceiling Tile		$9.91 \pm 5.32 \times 10^5$	$4.01 \pm 4.25 \times 10^3$	2.53 ± 0.43
			Carpet		$7.66 \pm 1.28 \times 10^7$	$0.94 \pm 1.23 \times 10^6$	2.14 ± 0.42
			Bare Pine Wood		$1.43 \pm 0.73 \times 10^6$	$8.31 \pm 4.34 \times 10^3$	2.24 ± 0.30
17	300/18	28/75	Glass	1.13×10^8	$6.12 \pm 2.11 \times 10^7$	$2.88 \pm 4.67 \times 10^5$	2.68 ± 0.53
			Ceiling Tile		$3.10 \pm 2.29 \times 10^6$	$1.78 \pm 1.49 \times 10^4$	2.33 ± 0.45
			Carpet		$9.60 \pm 1.88 \times 10^7$	$1.91 \pm 0.98 \times 10^6$	1.74 ± 0.21
			Bare Pine Wood		$7.35 \pm 6.29 \times 10^6$	$3.34 \pm 2.59 \times 10^4$	2.30 ± 0.48
18	300/60	27/45	Glass	1.10×10^8	$8.68 \pm 0.90 \times 10^7$	$1.62 \pm 0.32 \times 10^7$	0.73 ± 0.08
			Ceiling Tile		$3.98 \pm 0.72 \times 10^6$	$2.15 \pm 1.72 \times 10^3$	3.38 ± 0.33
			Carpet		$7.88 \pm 0.59 \times 10^7$	$1.53 \pm 0.55 \times 10^6$	1.73 ± 0.14
			Bare Pine Wood		$0.96 \pm 1.33 \times 10^7$	$8.28 \pm 4.66 \times 10^3$	2.88 ± 0.42
19	212/72	32/45	Glass	1.02×10^8	$7.32 \pm 1.20 \times 10^7$	$1.18 \pm 0.31 \times 10^7$	0.80 ± 0.12
			Ceiling Tile		$2.97 \pm 1.30 \times 10^6$	$1.64 \pm 1.68 \times 10^3$	3.46 ± 0.50
			Carpet		$9.06 \pm 2.70 \times 10^7$	$2.50 \pm 1.65 \times 10^6$	1.67 ± 0.40
			Bare Pine Wood		$5.81 \pm 3.66 \times 10^6$	$8.72 \pm 8.43 \times 10^4$	1.98 ± 0.63
20	300/60	32/45	Glass	1.03×10^8	$4.51 \pm 1.50 \times 10^7$	$1.56 \pm 0.08 \times 10^7$	0.44 ± 0.14
			Ceiling Tile		$3.33 \pm 2.15 \times 10^6$	$2.40 \pm 2.16 \times 10^3$	3.19 ± 0.43
			Carpet		$1.16 \pm 0.12 \times 10^8$	$2.50 \pm 3.07 \times 10^6$	1.89 ± 0.42
			Bare Pine Wood		$3.64 \pm 2.90 \times 10^6$	$3.62 \pm 1.87 \times 10^3$	2.96 ± 0.34

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).

Table A-4. Inactivation of *B. anthracis* Sterne Spores using Methyl Bromide[a]

Test Number	Target Parameters: Concentration (mg/L) / Contact Time (hr)	Target Parameters: Temp (°C) / RH (%)	Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* Sterne (CFU/coupon): Positive Control[b]	Mean Recovered *B.a.* Sterne (CFU/coupon): Test Coupon[c]	Decontamination Efficacy ± CI[d]
6	212/48	27/45	Glass	9.63×10^7	$9.82 \pm 3.80 \times 10^6$	0.00 ± 0.00	≥6.97 ± 0.15
			Ceiling Tile		$1.01 \pm 0.15 \times 10^7$	0.00 ± 0.00	≥7.00 ± 0.06
			Carpet		$7.46 \pm 0.39 \times 10^7$	0.00 ± 0.00	≥7.87 ± 0.02
			Painted Wallboard Paper		$3.89 \pm 0.82 \times 10^7$	0.00 ± 0.00	≥7.58 ± 0.07
			Bare Pine Wood		$6.95 \pm 1.85 \times 10^6$	$4.01 \pm 4.85 \times 10^3$	4.28 ± 1.53
			Unpainted Concrete		$8.55 \pm 6.09 \times 10^6$	0.00 ± 0.00	≥6.85 ± 0.26
7	212/24	27/75	Glass	1.00×10^8	$1.33 \pm 0.36 \times 10^7$	$0.75 \pm 1.08 \times 10^4$	4.17 ± 1.52
			Ceiling Tile		$9.25 \pm 1.15 \times 10^6$	$1.85 \pm 1.61 \times 10^4$	3.53 ± 1.70
			Carpet		$6.75 \pm 0.44 \times 10^7$	$2.87 \pm 1.70 \times 10^5$	2.47 ± 0.33
			Painted Wallboard Paper		$4.15 \pm 0.48 \times 10^7$	$6.73 \pm 3.44 \times 10^5$	1.86 ± 0.27
			Bare Pine Wood		$9.79 \pm 3.55 \times 10^6$	$1.72 \pm 1.83 \times 10^5$	1.88 ± 0.34
			Unpainted Concrete		$5.48 \pm 2.64 \times 10^6$	$0.94 \pm 1.66 \times 10^4$	3.83 ± 1.54
8	212/48	27/45	Glass	1.10×10^8	$1.28 \pm 0.28 \times 10^7$	$1.07 \pm 2.20 \times 10^2$	6.25 ± 1.08
			Ceiling Tile		$7.43 \pm 1.18 \times 10^6$	$2.52 \pm 4.31 \times 10^3$	3.99 ± 0.69
			Carpet		$6.18 \pm 1.11 \times 10^7$	0.00 ± 0.00	≥7.78 ± 0.07
			Painted Wallboard Paper		$3.73 \pm 0.78 \times 10^7$	0.00 ± 0.00	≥7.56 ± 0.08
			Bare Pine Wood		$9.37 \pm 1.67 \times 10^6$	$1.88 \pm 3.21 \times 10^4$	3.56 ± 0.98
			Unpainted Concrete		$3.87 \pm 2.08 \times 10^6$	0.00 ± 0.00	≥6.54 ± 0.21
9	212/36	27/75	Glass	9.63×10^7	$3.86 \pm 2.05 \times 10^7$	0.00 ± 0.00	≥7.53 ± 0.23
			Ceiling Tile		$1.15 \pm 0.16 \times 10^7$	0.00 ± 0.00	≥7.06 ± 0.06
			Carpet		$6.07 \pm 0.70 \times 10^7$	$2.06 \pm 2.93 \times 10^1$	7.11 ± 0.81
			Bare Pine Wood		$6.45 \pm 0.59 \times 10^6$	$0.75 \pm 1.44 \times 10^1$	6.50 ± 0.60
10	300/48	22/45	Glass	1.05×10^8	$1.27 \pm 0.41 \times 10^7$	$1.41 \pm 2.94 \times 10^1$	6.72 ± 0.73
			Ceiling Tile		$1.37 \pm 0.25 \times 10^7$	0.00 ± 0.00	≥7.13 ± 0.07
			Carpet		$7.49 \pm 1.70 \times 10^7$	$1.07 \pm 2.38 \times 10^2$	7.32 ± 1.07
			Bare Pine Wood		$1.15 \pm 0.23 \times 10^7$	$9.23 \pm 8.79 \times 10^3$	4.14 ± 1.65
11	212/60	22/45	Glass	1.00×10^8	$1.62 \pm 0.77 \times 10^7$	$2.91 \pm 6.20 \times 10^3$	5.01 ± 1.31
			Ceiling Tile		$1.35 \pm 0.38 \times 10^7$	$1.41 \pm 2.94 \times 10^1$	6.75 ± 0.72
			Carpet		$9.01 \pm 2.84 \times 10^7$	$3.56 \pm 5.27 \times 10^3$	5.25 ± 1.40
			Bare Pine Wood		$1.11 \pm 0.32 \times 10^7$	$1.18 \pm 1.74 \times 10^4$	3.35 ± 0.59
12	212/24	32/75	Glass	1.16×10^8	$8.00 \pm 2.03 \times 10^6$	0.00 ± 0.00	≥6.89 ± 0.09
			Ceiling Tile		$1.21 \pm 0.36 \times 10^7$	0.00 ± 0.00	≥7.07 ± 0.10
			Carpet		$6.24 \pm 0.87 \times 10^7$	0.00 ± 0.00	≥7.79 ± 0.06
			Bare Pine Wood		$7.16 \pm 2.60 \times 10^6$	0.00 ± 0.00	≥6.84 ± 0.12
13	212/48	32/45	Glass	9.30×10^7	$1.15 \pm 0.62 \times 10^7$	$4.70 \pm 2.63 \times 10^3$	3.40 ± 0.34
			Ceiling Tile		$9.76 \pm 2.00 \times 10^6$	$1.02 \pm 0.97 \times 10^3$	4.13 ± 0.38
			Carpet		$6.61 \pm 0.47 \times 10^7$	$2.73 \pm 3.60 \times 10^1$	7.09 ± 0.88
			Bare Pine Wood		$5.28 \pm 1.07 \times 10^6$	$1.11 \pm 0.92 \times 10^3$	3.95 ± 0.59

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).

Table A-4. Inactivation of *B. anthracis* Sterne Spores using Methyl Bromide[a] *(Continued)*

Test Number	Target Parameters: Concentration (mg/L) / Contact Time (hr)	Target Parameters: Temp (°C) / RH (%)	Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* Sterne (CFU/coupon): Positive Control[b]	Mean Recovered *B.a.* Sterne (CFU/coupon): Test Coupon[c]	Decontamination Efficacy ± CI[d]
14	300/24	22/75	Glass	1.06×10^8	$9.60 \pm 3.37 \times 10^6$	$3.02 \pm 2.11 \times 10^4$	2.57 ± 0.30
			Ceiling Tile		$2.59 \pm 0.74 \times 10^7$	$7.10 \pm 4.42 \times 10^5$	1.61 ± 0.25
			Carpet		$7.57 \pm 1.30 \times 10^7$	$7.56 \pm 5.91 \times 10^5$	2.10 ± 0.30
			Bare Pine Wood		$1.14 \pm 0.18 \times 10^7$	$2.54 \pm 1.75 \times 10^5$	1.72 ± 0.25
15	300/60	22/45	Glass	1.18×10^8	$1.04 \pm 0.49 \times 10^7$	0.00 ± 0.00	≥6.98 ± 0.18
			Ceiling Tile		$2.74 \pm 0.99 \times 10^7$	$1.82 \pm 1.49 \times 10^3$	4.79 ± 1.32
			Carpet		$8.69 \pm 0.79 \times 10^7$	$2.06 \pm 2.93 \times 10^1$	7.27 ± 0.81
			Bare Pine Wood		$1.53 \pm 0.62 \times 10^7$	$3.86 \pm 2.41 \times 10^3$	3.67 ± 0.34
16	212/60	32/45	Glass	1.22×10^8	$1.01 \pm 1.07 \times 10^7$	$2.85 \pm 5.75 \times 10^4$	4.21 ± 1.78
			Ceiling Tile		$1.02 \pm 0.09 \times 10^7$	$1.50 \pm 2.15 \times 10^3$	4.63 ± 1.26
			Carpet		$6.40 \pm 0.66 \times 10^7$	0.00 ± 0.00	≥7.80 ± 0.04
			Bare Pine Wood		$9.53 \pm 2.46 \times 10^6$	$3.95 \pm 4.22 \times 10^3$	4.15 ± 1.43
17	300/18	27/75	Glass	9.97×10^7	$2.15 \pm 1.17 \times 10^7$	$7.73 \pm 3.59 \times 10^2$	4.41 ± 0.32
			Ceiling Tile		$1.33 \pm 0.32 \times 10^7$	$6.82 \pm 4.56 \times 10^4$	2.40 ± 0.37
			Carpet		$1.07 \pm 0.19 \times 10^8$	$1.62 \pm 1.65 \times 10^4$	4.38 ± 1.09
			Bare Pine Wood		$1.63 \pm 1.06 \times 10^7$	$2.33 \pm 2.24 \times 10^4$	3.04 ± 0.58
18	300/60	27/45	Glass	1.07×10^8	$7.55 \pm 2.61 \times 10^6$	$2.71 \pm 2.74 \times 10^1$	5.88 ± 0.80
			Ceiling Tile		$1.09 \pm 0.22 \times 10^7$	$3.58 \pm 2.86 \times 10^3$	3.60 ± 0.33
			Carpet		$5.81 \pm 1.03 \times 10^7$	$2.74 \pm 5.90 \times 10^1$	7.33 ± 0.84
			Bare Pine Wood		$1.07 \pm 0.27 \times 10^7$	$4.31 \pm 9.38 \times 10^4$	3.67 ± 1.04
19	212/72	32/45	Glass	1.01×10^8	$1.13 \pm 0.32 \times 10^7$	$1.01 \pm 1.60 \times 10^2$	6.10 ± 1.14
			Ceiling Tile		$9.74 \pm 2.51 \times 10^6$	$0.89 \pm 1.43 \times 10^3$	5.27 ± 1.45
			Carpet		$7.69 \pm 1.08 \times 10^7$	0.00 ± 0.00	≥7.88 ± 0.05
			Bare Pine Wood		$6.40 \pm 1.44 \times 10^6$	$4.36 \pm 2.93 \times 10^3$	3.41 ± 0.64
20	300/60	32/45	Glass	1.06×10^8	$1.71 \pm 1.52 \times 10^7$	$3.41 \pm 5.06 \times 10^3$	3.95 ± 0.58
			Ceiling Tile		$1.07 \pm 0.27 \times 10^7$	$1.91 \pm 2.60 \times 10^3$	4.02 ± 0.47
			Carpet		$5.78 \pm 0.82 \times 10^7$	$0.75 \pm 1.44 \times 10^1$	7.45 ± 0.60
			Bare Pine Wood		$5.93 \pm 0.90 \times 10^6$	$7.08 \pm 4.55 \times 10^3$	3.01 ± 0.31

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).

Appendix B
Comparing Efficacy for the Different Microorganisms

Testing was first conducted using *G.s.* as a potential surrogate for *B.a.* Ames (Tests 1 & 2). The results showed that *G.s.* is less resistant than *B.a.* Ames to MeBr exposure; therefore, the additional potential surrogates, *B.a.* NNR1Δ1 and *B.a.* Sterne, were tested. In an attempt to evaluate all three organisms at once, the number of coupon materials tested was reduced due to the size of the MeBr test chamber. Painted wallboard and unpainted concrete were removed from testing after Test 8 as these materials were the easiest to decontaminate. The detailed differences in efficacy by material type and test number are shown in Tables B-1 and B-2.

Table B-1. Difference in MeBr Efficacy between *B. anthracis* Ames and *G. stearothermophilus**

Test Number	Target MeBr Concentration (mg/L)	Target Temperature (°C)	Target RH (%)	Contact Time (hour)	Material Type	*B.a.* Ames Efficacy	*G.s.* Efficacy	Average Difference in Efficacy
1	212	22	45	36	Glass	3.14	≥ 7.72	2.26
					Ceiling Tile	3.47	4.89	
					Carpet	4.18	6.77	
					Painted Wallboard Paper	≥ 7.04	≥ 7.82	
					Pine Wood	2.16	≥ 5.75	
					Unpainted Concrete	≥ 6.17	6.78	
2	212	22	45	48	Glass	3.00	≥ 7.76	1.10
					Ceiling Tile	≥ 6.76	≥ 5.00	
					Carpet	6.35	≥ 7.19	
					Painted Wallboard Paper	≥ 7.21	≥ 7.43	
					Pine Wood	2.82	≥ 5.37	
					Unpainted Concrete	≥ 6.89	≥ 6.85	

* Results shown as efficacy (log reduction).

Table B-2. Difference in MeBr Efficacy between *B. anthracis* Ames, *B. anthracis* NNR1Δ1, and *B. anthracis* Sterne*

Test Number	Target MeBr Concentration (mg/L)	Target Temperature (°C)	Target RH (%)	Contact Time (hour)	Material Type	*B.a.* Ames Efficacy	*B.a.* NNR1Δ1 Efficacy	Average Difference in Efficacy	*B.a.* Sterne Efficacy	Average Difference in Efficacy
3	212	22	75	36	Glass	≥ 7.92	0.60	-6.33	NT	NT
					Ceiling Tile	≥ 7.07	1.12			
					Carpet	≥ 7.93	1.56			
					Painted Wallboard Paper	≥ 7.76	0.88			
					Pine Wood	≥ 7.02	1.80			
					Unpainted Concrete	≥ 7.51	1.26			
4	212	27	45	36	Glass	4.86	1.97	-1.29	NT	NT
					Ceiling Tile	6.41	5.57			
					Carpet	≥ 7.95	4.89			
					Painted Wallboard Paper	≥ 7.50	≥ 7.77			
					Pine Wood	5.93	5.08			
					Unpainted Concrete	≥ 7.23	≥ 6.88			
5	212	22	75	24	Glass	2.54	0.35	-2.20	NT	NT
					Ceiling Tile	2.70	0.80			
					Carpet	3.31	0.68			
					Painted Wallboard Paper	2.69	0.61			
					Pine Wood	2.99	0.84			
					Unpainted Concrete	3.41	1.16			
6	212	27	45	48	Glass	3.49	NT	NT	≥ 6.97	1.74
					Ceiling Tile	4.00			≥ 7.00	
					Carpet	6.60			≥ 7.87	
					Painted Wallboard Paper	≥ 7.34			≥ 7.58	
					Pine Wood	1.86			4.28	
					Unpainted Concrete	≥ 6.84			≥ 6.85	
7	212	27	75	24	Glass	4.42	NT	NT	4.17	-3.75
					Ceiling Tile	≥ 7.14			3.53	
					Carpet	7.40			2.47	
					Painted Wallboard Paper	≥ 7.79			1.86	
					Pine Wood	6.29			1.88	
					Unpainted Concrete	≥ 7.17			3.83	
8	212	27	45	48	Glass	2.29	NT	NT	6.25	1.54
					Ceiling Tile	3.29			3.99	
					Carpet	4.31			≥ 7.78	
					Painted Wallboard Paper	≥ 7.60			≥ 7.56	
					Pine Wood	2.03			3.56	
					Unpainted Concrete	≥ 6.94			≥ 6.54	

* Results shown as efficacy (log reduction).
NT = Not Tested

Table B-2. Difference in MeBr Efficacy between *B. anthracis* Ames, *B. anthracis* NNR1Δ1, and *B. anthracis* Sterne* *(Continued)*

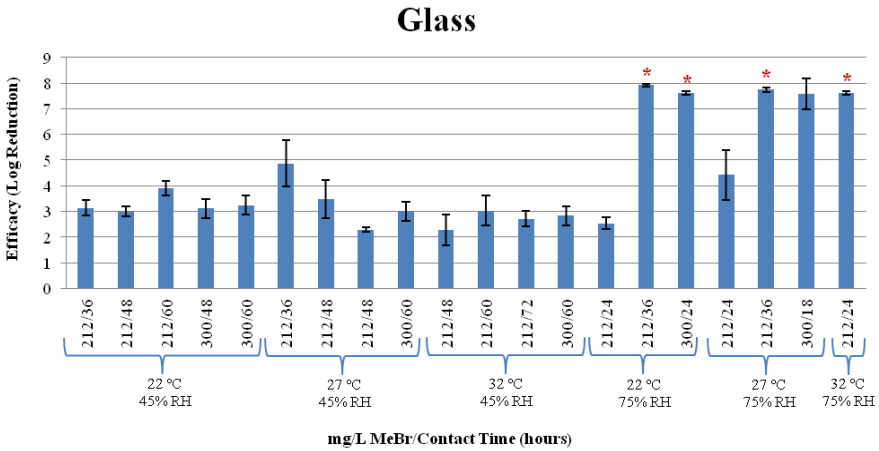
Test Number	Target MeBr Concentration (mg/L)	Target Temperature (°C)	Target RH (%)	Contact Time (hour)	Material Type	*B.a.* Ames Efficacy	*B.a.* NNR1Δ1 Efficacy	Average Difference in Efficacy	*B.a.* Sterne Efficacy	Average Difference in Efficacy
9	212	27	75	36	Glass	≥ 7.74	2.79	-1.83	≥ 7.53	-0.39
					Ceiling Tile	≥ 7.07	≥ 6.48		≥ 7.06	
					Carpet	≥ 8.04	≥ 7.91		7.11	
					Bare Pine Wood	≥ 6.92	5.26		6.50	
10	300	22	45	48	Glass	3.11	0.62	-1.55	6.72	1.86
					Ceiling Tile	≥ 7.23	6.29		≥ 7.13	
					Carpet	4.60	1.65		7.32	
					Bare Pine Wood	2.95	3.13		4.14	
11	212	22	45	60	Glass	3.90	0.87	-1.63	5.01	0.72
					Ceiling Tile	≥ 7.16	5.33		6.75	
					Carpet	3.41	1.30		5.25	
					Bare Pine Wood	3.03	3.48		3.35	
12	212	32	75	24	Glass	≥ 7.61	1.73	-2.54	≥ 6.89	-0.22
					Ceiling Tile	≥ 7.04	≥ 6.64		≥ 7.07	
					Carpet	≥ 7.91	7.25		≥ 7.79	
					Bare Pine Wood	≥ 6.89	3.69		≥ 6.84	
13	212	32	45	48	Glass	2.28	0.60	-1.59	3.40	1.31
					Ceiling Tile	3.93	2.67		4.13	
					Carpet	4.65	1.63		7.09	
					Bare Pine Wood	2.47	2.06		3.95	
14	300	22	75	24	Glass	≥ 7.62	1.03	-6.16	2.57	-5.35
					Ceiling Tile	6.72	1.36		1.61	
					Carpet	≥ 8.02	0.75		2.10	
					Bare Pine Wood	≥ 7.04	1.63		1.72	
15	300	22	45	60	Glass	3.25	0.87	-1.03	≥ 6.98	1.61
					Ceiling Tile	5.36	5.87		4.79	
					Carpet	5.30	1.85		7.27	
					Bare Pine Wood	2.38	3.60		3.67	
16	212	32	45	60	Glass	3.03	0.48	-1.92	4.21	1.43
					Ceiling Tile	4.36	2.53		4.63	
					Carpet	5.29	2.14		≥ 7.80	
					Bare Pine Wood	2.40	2.24		4.15	
17	300	27	75	18	Glass	7.58	2.68	-4.96	4.41	-3.67
					Ceiling Tile	6.29	2.33		2.40	
					Carpet	≥ 8.11	1.74		4.38	
					Bare Pine Wood	≥ 6.92	2.30		3.04	
18	300	27	45	60	Glass	3.01	0.73	-1.66	5.88	1.28
					Ceiling Tile	4.36	3.38		3.60	
					Carpet	5.28	1.73		7.33	
					Bare Pine Wood	2.71	2.88		3.67	
19	212	32	45	72	Glass	2.72	0.80	-2.06	6.10	1.63
					Ceiling Tile	5.20	3.46		5.27	
					Carpet	6.02	1.67		≥ 7.88	
					Bare Pine Wood	2.21	1.98		3.41	
20	300	32	45	60	Glass	2.84	0.44	-1.51	3.95	0.98
					Ceiling Tile	3.60	3.19		4.02	
					Carpet	5.82	1.89		7.45	
					Bare Pine Wood	2.27	2.96		3.01	

* Results shown as efficacy (log reduction).

Appendix C
Effects of Materials and Operational Parameters on MeBr Efficacy

Effects of Test Materials on MeBr efficacy

Testing was originally conducted using six test materials (Tests 1 through 8): ceiling tile, carpet, glass, painted wallboard paper, bare pine wood, and unpainted concrete. In an attempt to evaluate three organisms at once (*B.a.* Ames, *B.a.* NNR1Δ1, and *B.a.* Sterne), the number of coupon materials tested was reduced due to the size of the MeBr test chamber. Painted wallboard and unpainted concrete were removed from testing in Tests 9 through 20, as these two material types generally exhibited higher efficacy than the other material types. A summary of the results in terms of LR are organized by operational parameters and can be seen in Figures C-1 through C-3 below.



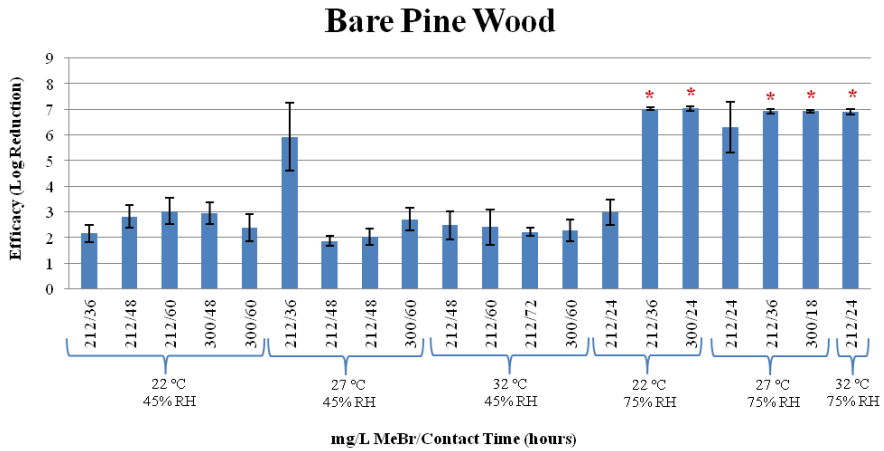
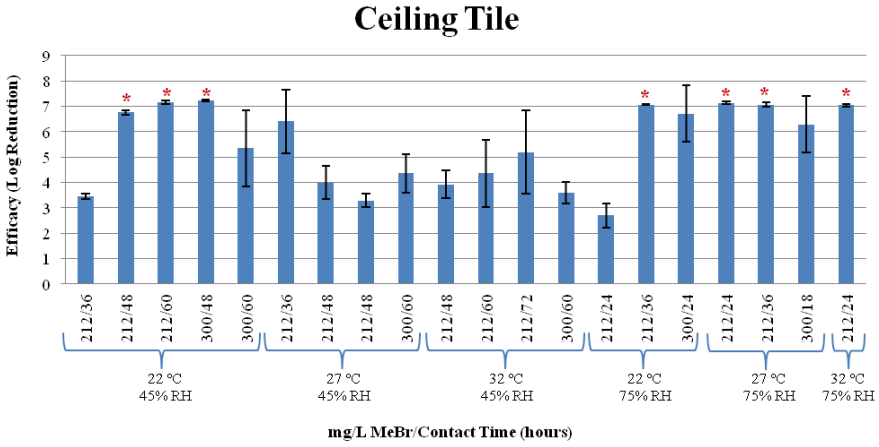


Figure C-1. Summary of MeBr efficacy against *B. anthracis* Ames on glass and bare pine wood. Results shown in average log reduction ± CI.

*** Complete inactivation achieved**



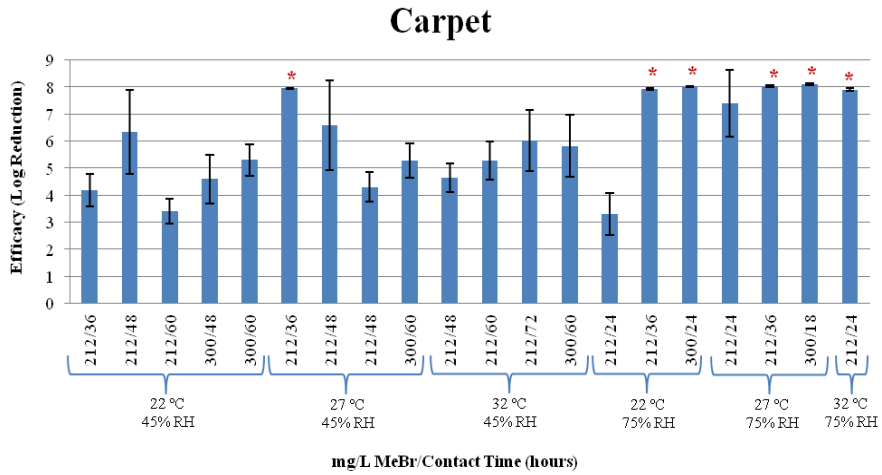


Figure C-2. Summary of MeBr efficacy against *B. anthracis* Ames on ceiling tile and carpet. Results shown in average log reduction ± CI.

*** Complete inactivation achieved**

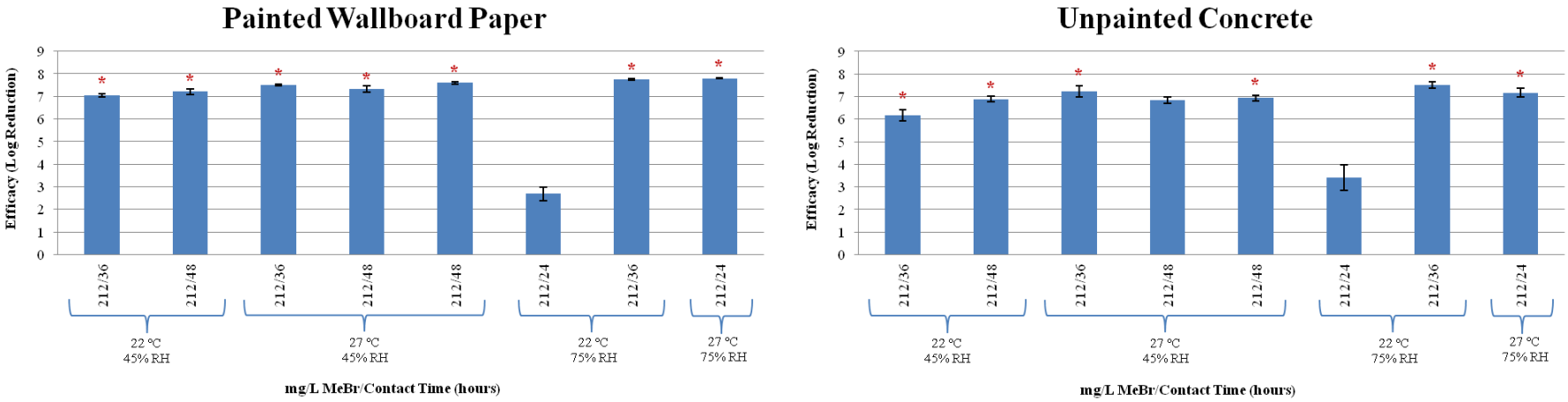


Figure C-3. Summary of MeBr efficacy against *B. anthracis* Ames on painted wallboard paper and unpainted concrete. Results shown in average log reduction ± CI.

*** Complete inactivation achieved**

Effects of Temperature on MeBr Efficacy against *B.a.* Ames

The decontamination efficacy of MeBr against *B.a*. Ames was evaluated at target temperatures of 22, 27, or 32 °C. These temperatures were tested at various combinations of %RH, MeBr concentration, and contact time and results are summarized in Tables C-1 and C-2. The comparisons are made for two test conditions which share the same fumigation parameters except temperature.

Table C-1. Effect of Increasing Temperature at Low Relative Humidity on MeBr Efficacy*

Material Type	Test 1[a] 212 mg/L; 22 °C; 45 %; 36 hr	Test 4 212 mg/L; 27 °C; 45 %; 36 hr	Average Difference in Efficacy	Test 2 212 mg/L; 22 °C; 45 %; 48 hr	Test 6 212 mg/L; 27 °C; 45 %; 48 hr	Average Difference in Efficacy
Glass	3.14	4.86		3.00	3.49	
Ceiling Tile	3.47	6.41		≥ 6.76	4.00	
Carpet	4.18	≥ 7.95	2.29	6.35	6.60	-0.48
Painted Wallboard Paper	≥ 7.04	≥ 7.50		≥ 7.21	7.34	
Bare Pine Wood	2.16	5.93		2.82	1.86	
Unpainted Concrete	≥ 6.17	≥ 7.23		≥ 6.89	6.84	

Material Type	Test 6 212 mg/L; 27 °C; 45 %; 48 hr	Test 13 212 mg/L 32 °C 45 % 48 hr	Average Difference in Efficacy	Test 11 212 mg/L; 22 °C; 45 %; 60 hr	Test 16 212 mg/L; 32 °C; 45 %; 60 hr	Average Difference in Efficacy
Glass	3.49	2.28		3.90	3.03	
Ceiling Tile	4.00	3.93		≥ 7.16	4.36	
Carpet	6.60	4.65	-0.66	3.41	5.29	-0.61
Painted Wallboard Paper	≥ 7.34	--[b]		--	--	
Bare Pine Wood	1.86	2.47		3.03	2.40	
Unpainted Concrete	≥ 6.84	--		--	--	

Material Type	Test 15 300 mg/L; 22 °C; 45 %; 60 hr	Test 18 300 mg/L 27 °C 45 % 60 hr	Average Difference in Efficacy	Test 18 300 mg/L; 27 °C; 45 %; 60 hr	Test 20 300 mg/L; 32 °C; 45 %; 60 hr	Average Difference in Efficacy
Glass	3.25	3.01		3.01	2.84	
Ceiling Tile	5.36	4.36		4.36	3.60	
Carpet	5.30	5.28	-0.23	5.28	5.82	-0.21
Painted Wallboard Paper	--	--		--	--	
Bare Pine Wood	2.38	2.71		2.71	2.27	
Unpainted Concrete	--	--		--	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

Table C-2. Effect of Increasing Temperature at High Relative Humidity on MeBr Efficacy*

Material Type	Test 5[a] 212 mg/L; 22 °C; 75 %; 24 hr	Test 7 212 mg/L; 27 °C; 75 %; 24 hr	Average Difference in Efficacy	Test 3 212 mg/L; 22 °C; 75 %; 36 hr	Test 9 212 mg/L; 27 °C; 75 %; 36 hr	Average Difference in Efficacy
Glass	2.54	4.42	3.76	≥ 7.92	≥ 7.74	0.00
Ceiling Tile	2.70	≥ 7.14		≥ 7.07	≥ 7.07	
Carpet	3.31	7.40		≥ 7.93	≥ 8.04	
Painted Wallboard Paper	2.69	≥ 7.79		≥ 7.76	--[b]	
Bare Pine Wood	2.99	6.29		≥ 7.02	≥ 6.92	
Unpainted Concrete	3.41	≥ 7.17		≥ 7.51	--	

Material Type	Test 7 212 mg/L; 27 °C; 75 %; 24 hr	Test 12 212 mg/L; 32 °C; 75 %; 36 hr	Average Difference in Efficacy
Glass	4.42	≥ 7.61	1.05
Ceiling Tile	≥ 7.14	≥ 7.04	
Carpet	7.40	≥ 7.91	
Painted Wallboard Paper	≥ 7.79	--	
Bare Pine Wood	6.29	≥ 6.89	
Unpainted Concrete	≥ 7.17	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

Effect of Relative Humidity on Efficacy of MeBr against *B. anthracis* Ames

The decontamination efficacy of MeBr against *B.a.* Ames was evaluated at target relative humidities of 45 or 75 %. The actual %RH conditions for each test are shown in Appendix A. These RH levels were tested at various temperatures, MeBr concentrations, and contact times and results are summarized in Table 6-5 below and discussed in Section 6.4. The comparisons are made for two test conditions which share the same fumigation parameters except RH.

Table C-3. Effect of Increasing Relative Humidity at Low and High Temperatures on *B. anthracis* Ames*

Material Type	Test 1[a] 212 mg/L; 22 °C; 45%; 36 hr	Test 3 212 mg/L; 22 °C; 75%; 36 hr	Average Difference in Efficacy	Test 4 212 mg/L; 27 °C; 45%; 36 hr	Test 9 212 mg/L; 27 °C; 75%; 36 hr	Average Difference in Efficacy
Glass	3.14	≥ 7.92	3.18	4.86	≥ 7.74	1.16
Ceiling Tile	3.47	≥ 7.07		6.41	≥ 7.07	
Carpet	4.18	≥ 7.93		≥ 7.95	≥ 8.04	
Painted Wallboard Paper	≥ 7.04	≥ 7.76		≥ 7.50	--[b]	
Bare Pine Wood	2.16	≥ 7.02		5.93	≥ 6.92	
Unpainted Concrete	≥ 6.17	≥ 7.51		≥ 7.23	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

Effects of MeBr Concentration on Efficacy against *B. anthracis* Ames

The decontamination efficacy of MeBr against virulent *B.a.* was also evaluated at target concentrations of 212 and 300 mg/L. These concentrations were tested in various combinations of temperature and RH. The results are summarized in Tables C-4 and C-5, below. The comparisons are made for two test conditions that share the same fumigation parameters except MeBr concentration.

Table C-4. Effect of Increasing MeBr Concentration at Low Relative Humidity on *B. anthracis* Ames*

Material Type	Test 2[a] 212 mg/L; 22 °C; 45%; 48 hr	Test 10 212 mg/L; 22 °C; 45%; 48 hr	Average Difference in Efficacy	Test 11 212 mg/L; 22 °C; 45%; 60 hr	Test 15 300 mg/L; 22 °C; 45%; 60 hr	Average Difference in Efficacy
Glass	3.00	3.11		3.90	3.25	
Ceiling Tile	≥ 6.76	≥ 7.23		≥ 7.16	5.36	
Carpet	6.35	4.60	-0.26	3.41	5.30	-0.30
Painted Wallboard Paper	≥ 7.21	--[b]		--	--	
Bare Pine Wood	2.82	2.95		3.03	2.38	
Unpainted Concrete	≥ 6.89	--		--	--	

Material Type	Test 16 212 mg/L; 32 °C; 45%; 60 hr	Test 20 300 mg/L; 32 °C; 45%; 60 hr	Average Difference in Efficacy
Glass	3.03	2.84	
Ceiling Tile	4.36	3.60	
Carpet	5.29	5.82	-0.14
Painted Wallboard Paper	--	--	
Bare Pine Wood	2.40	2.27	
Unpainted Concrete	--	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

Table C-5. Effect of Increasing MeBr Concentration at High Relative Humidity on *B. anthracis* Ames*

Material Type	Test 5[a] 212 mg/L; 22 °C; 75 %; 24 hr	Test 14 300 mg/L; 22 °C; 75 %; 24 hr	Average Difference in Efficacy
Glass	2.54	≥ 7.62	
Ceiling Tile	2.70	6.72	
Carpet	3.31	≥ 8.02	4.47
Painted Wallboard Paper	2.69	--[b]	
Bare Pine Wood	2.99	≥ 7.04	
Unpainted Concrete	3.41	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

Effects of Contact Time on Efficacy of MeBr against *B. anthracis* Ames

The effect of increasing the contact times to MeBr at low and high %RH on the efficacy against *B.a.* Ames was also assessed. The contact times tested ranged from 18 to 72 hours and actual contact times did not deviate from these targets. The results are summarized in Tables C-6 and C-7 and Figure 6-6. The comparisons are made for two test conditions that share the same fumigation parameters except contact time.

Table C-6. Effect of Increasing Contact Time at Low Relative Humidity on *B. anthracis* Ames*

Material Type	Test 1[a] 212 mg/L; 22 °C; 45 %; 36 hr	Test 2 212 mg/L; 22 °C; 45 %; 48 hr	Average Difference in Efficacy	Test 2 212 mg/L; 22 °C; 45 %; 48 hr	Test 11 212 mg/L; 22 °C; 45 %; 60 hr	Average Difference in Efficacy
Glass	3.14	3.00		3.00	3.90	
Ceiling Tile	3.47	≥ 6.76		≥ 6.76	≥ 7.16	
Carpet	4.18	6.35	1.15	6.35	3.41	-0.36
Painted Wallboard Paper	≥ 7.04	≥ 7.21		≥ 7.21	--[b]	
Bare Pine Wood	2.16	2.82		2.82	3.03	
Unpainted Concrete	≥ 6.17	≥ 6.89		≥ 6.89	--	

Material Type	Test 4 212 mg/L; 27 °C; 45 %; 36 hr	Test 6 212 mg/L; 27 °C; 45 %; 48 hr	Average Difference in Efficacy	Test 13 212 mg/L; 32 °C; 45 %; 48 hr	Test 16 212 mg/L; 32 °C; 45%; 60 hr	Average Difference in Efficacy
Glass	4.86	3.49		2.28	3.03	
Ceiling Tile	6.41	4.00		3.93	4.36	
Carpet	≥ 7.95	6.60	-1.63	4.65	5.29	0.44
Painted Wallboard Paper	≥ 7.50	≥ 7.34		--	--	
Bare Pine Wood	5.93	1.86		2.47	2.40	
Unpainted Concrete	≥ 7.23	≥ 6.84		--	--	

Material Type	Test 16 212 mg/L; 32 °C; 45 %; 60 hr	Test 19 212 mg/L; 32 °C; 45 %; 72 hr	Average Difference in Efficacy	Test 10 300 mg/L; 22 °C; 45 %; 48 hr	Test 15 300 mg/L; 22 °C; 45 %; 60 hr	Average Difference in Efficacy
Glass	3.03	2.72		3.11	3.25	
Ceiling Tile	4.36	5.20		≥ 7.23	5.36	
Carpet	5.29	6.02	0.27	4.60	5.30	-0.40
Painted Wallboard Paper	--	--		--	--	
Bare Pine Wood	2.40	2.21		2.95	2.38	
Unpainted Concrete	--	--		--	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

Table C-7. Effect of Increasing Contact Time at High Relative Humidity on *B. anthracis* Ames*

Material Type	Test 5[a] 212 mg/L; 22 °C; 75 %; 24 hr	Test 3 212 mg/L; 22 °C; 75 %; 36 hr	Average Difference in Efficacy	Test 7 212 mg/L; 27 °C; 75 %; 24 hr	Test 9 212 mg/L; 27 °C; 75 %; 36 hr	Average Difference in Efficacy
Glass	2.54	≥ 7.92	4.60	4.42	≥ 7.74	1.13
Ceiling Tile	2.70	≥ 7.07		≥ 7.14	≥ 7.07	
Carpet	3.31	≥ 7.93		7.40	≥ 8.04	
Painted Wallboard Paper	2.69	≥ 7.76		≥ 7.79	--[b]	
Bare Pine Wood	2.99	≥ 7.02		6.29	≥ 6.92	
Unpainted Concrete	3.41	≥ 7.51		≥ 7.17	--	

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MeBr concentration (mg/L), temperature (°C), %RH, and contact time (hrs).
[b] "--" Not tested.

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