EPA/600/R-14/208 | August 2014 | www.epa.gov/ord

United States
Environmental Protection
Agency

Decontamination of a Mock Office Using Chlorine Dioxide Gas

Office of Research and Development
National Homeland Security Research Center

EPA/600/R-14/208
August 2014

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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's (ORD's) National Homeland Security Research Center (NHSRC), funded and directed this work through Contract Number EP-C-09-027 with ARCADIS Inc. This report has been peer and administratively reviewed and has been approved for publication as an EPA document. The views expressed in this report are those of the authors and do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

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Acknowledgments

Contributions of the following individuals and organization to this report are gratefully acknowledged:

United States Environmental Protection Agency (EPA)

Worth Calfee
Shawn Ryan

Peer reviewers

Marissa Mullins, EPA Office of Emergency Management
Eugene Rice, EPA Office of Research and Development, National Homeland Security Research Center
Doris Betancourt, EPA Office of Research and Development, National Risk Management Research Laboratory

ARCADIS

Executive Summary

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. As part of the HSRP, EPA is investigating the effectiveness and applicability of technologies for homeland security (HS)-related applications. The purpose of this investigation was to determine the efficacy of using relatively low levels of chlorine dioxide (ClO_2) gas for inactivating *Bacillus anthracis* (causative agent for anthrax) surrogate spores in an office environment. Previous tests and actual fumigations using high levels of ClO_2 (e.g., 1000 – 3000 parts per million) have demonstrated its efficaciousness, but the use of high ClO_2 levels also comes with drawbacks such as issues with material compatibility and generation technology capacity.

Demonstration of successful decontamination efficacy with ClO_2 gas at concentrations lower than what has been used previously would allow for a greater number of vendors to participate in remediation efforts following a large anthrax spore release. That is, vendors with technologies that produce ClO_2, but perhaps at relatively lower generation rates, would still be able to meet the target fumigation concentration within a large building. The objective of this study was to provide an understanding of the performance (i.e., efficacy) of low level ClO_2 gas to guide its use and implementation in HS applications for hard-to-decontaminate materials such as those found in an office. In the assessment of options for decontamination following an intentional release of *B. anthracis* spores, it is important to know what operational factors can impact the decontamination efficacy.

This investigation initially focused on decontamination tests at bench scale using small coupons inoculated with spores of *B. subtilis* and then fumigated inside a glove box. These small scale tests were then followed by pilot-scale tests using a mock office set up within a large decontamination chamber. Tests were conducted with varying operational parameters (e.g., ClO_2 concentration and contact time), to assess the effect of these parameters on decontamination efficacy. Various types of microbiological assays were used extensively to characterize spore levels before and after treatment with low level ClO_2, including surface sampling, biological indicators (BIs), bioaerosol measurements, reference measurement coupons, and others.

Thirteen pilot-scale tests were conducted with spores of *B. atrophaeus*, a microorganism previously verified as an appropriate surrogate for *B. anthracis* in ClO_2 decontamination studies. Spores were disseminated into the mock office as a dry powder. The effectiveness of the low level ClO_2 treatment was characterized in a number of ways, e.g., in terms of the number of spores recovered from surfaces following decontamination; the number of samples in which spores were not detected; and in terms of log reduction (LR). LR was determined based on the difference between the number of bacterial spores (as colony forming units, or CFU) recovered from the office before and after decontamination. In five of the tests, computers were included within the mock office to assess the impact of low level ClO_2 fumigation on sensitive electronics.

Summary of Results

Small scale tests

Tests were conducted in a glove box with ClO_2 concentrations of either 100 or 200 ppm, with contact times ranging from 2-12 hours. The coupon materials used were wood, concrete, carpet,

painted drywall paper, and galvanized metal. The results showed that low level ClO_2 fumigation was effective (> 6 LR) for nearly every condition tested except for the wood coupons at 200 ppm (although all LR results were greater than 5). In the majority of the tests, greater than 7 LR was obtained and/or all spores were completely inactivated. In every test with concrete and painted drywall paper, the spores were completely inactivated (no viable spores were detected), while for wood, there were no tests in which spores were completely inactivated.

Mock office efficacy results

The majority of the mock office tests used relatively low levels of ClO_2 gas (100-300 ppm), although a few tests were conducted at 750 ppm or higher to allow for comparison of results from previous studies. Contact times ranged from 3-24 hours. The RH was generally well controlled, and with the exception of one test, ranged from 74-78 %. Actual average temperatures for the tests ranged from 22-29 °C (72-84 °F).

The average spore loadings for the mock office, prior to decontamination, ranged from 4.89 – 7.21 log CFU/square foot (ft^2; 77,625 – 16.2 million CFU/ft^2). Overall average spore loadings for the office following ClO_2 treatment ranged from -0.01 to 2.24 log CFU/ft^2 (1 – 174 CFU/ft^2), and generally correlated (0.62) with pre-decontamination spore loadings. The post-decontamination spore loading correlation with pre-decontamination loading was apparent for specific surfaces within the office, as well as for the average loadings for the entire office. For example, Test 4, with only a 228 ppm ClO_2 average level and 4 hour contact time, resulted in highest number of post-decontamination surface samples in which no spores were detected (96%) for the study, but also had the second lowest average office spore loading prior to decontamination. Post-decontamination spore levels in the office air ranged from approximately 1 to 80 CFU/cubic meter (m^3). Additionally, post-decontamination average spore surface levels were well correlated (0.74) with the post-decontamination spore levels in the air.

The study demonstrates that decontamination efficacy levels between 5 to > 6 LR are achievable using relatively low levels of ClO_2, depending on the pre-decontamination spore levels and other factors. While none of the mock office tests resulted in overall average office LR values of greater than 6, there were several office tests in which spores were not detected from nearly all of the surfaces sampled, with corresponding LR levels > 6. Although the results for the small scale coupon tests are comparable to the mock office tests, it is difficult to make direct comparisons for the two scales of testing. Differences in materials, spore deposition methods, spore loadings, sampling procedures, surface sample size, etc., make direct comparisons difficult since these could affect efficacy results.

Biological indicators (BIs) were one of the assays used to assess effectiveness of the ClO_2 fumigation in inactivating bacterial spores. The BIs were comprised of nominally 10^6 spores of *B. atrophaeus* inoculated onto stainless steel discs and wrapped in Tyvek envelopes. The BIs were placed at five locations within the office and collected after fumigation and analyzed to determine whether any of the BIs exhibited growth of bacteria, i.e., survival of any spores. The results showed that every BI exposed to ClO_2 in the mock office tests was inactivated. This general result is consistent with other tests that have shown that the spore populations on BIs are typically much easier to inactivate than spores associated with coupons from building materials or actual environmental surfaces.

Impact on computer functionality

Fumigation at approximately 200 ppm ClO_2 for 6 or 8 hours and at approximately 300 ppm ClO_2 for 4 hours showed no measureable change in the function of the computers over the course of a year.

Implications of study

The study has demonstrated the potential of using relatively low levels of ClO_2 gas, accompanied by longer contact times, for effective decontamination of surfaces and spaces contaminated by anthrax spores. However, this decontamination approach may be better suited for areas that are not heavily contaminated, i.e., that have spore loadings less than 5 log CFU/ft^2 and/or that do not contain significant quantities of porous materials such as carpet and wood. Further research is recommended to find efficacious low concentration conditions for heavily contaminated surfaces and for difficult to decontaminate materials.

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Abbreviations/Acronyms

AKA	Also known as
ATCC	American Type Culture Collection
B. anthracis	*Bacillus anthracis* (Ames strain)
B. atrophaeus	*Bacillus atrophaeus*
BI	Biological Indicator
BOTE	Bio-Response Operational Testing and Evaluation
B. subtilis	*Bacillus subtilis* (ATCC 19659)
°C	degree(s) Celsius
CFU	colony forming unit(s)
ClO_2	chlorine dioxide
cm	centimeter(s)
COMMANDER	Consequence Management and Decontamination Evaluation Room
DAS	Digital Acquisition System
ELPI	Electrical Low Pressure Impactor
EPA	U.S. Environmental Protection Agency
FB	field blank
ft	feet/foot
g	gram(s)
H_2O_2	hydrogen peroxide
HEPA	High efficiency particulate air
hr	hour(s)
HS	homeland security
HSRP	Homeland Security Research Program
L	liter(s)
LR	Log_{10} reduction
m^3	cubic meter(s)
mm	millimeter
MDI	Metered Dose Inhaler
mg	milligram(s)
min	minute
mL	milliliter(s)
µL	microliter(s)
ND	not detected
NHSRC	National Homeland Security Research Center
ORD	Office of Research and Development
PBST	phosphate buffered saline with Tween
PCI	Peripheral Component Interconnect
ppm	part(s) per million
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
RH	relative humidity
RMC	Reference Material Coupon

SCADA	Supervisory Control and Data Acquisition
TSA	Triptic Soy Agar
USB	Universal Serial Bus
VHP®	Vaporized Hydrogen Peroxide ®

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 in buildings, water systems, or the outdoor environment; contain these contaminants; decontaminate buildings, water systems or the outdoor environment; and facilitate the treatment and disposal of materials resulting from remediation activities. As part of the above effort, and in response to the needs of the HSRP's Program Office partners, EPA investigates the effectiveness and applicability of technologies for homeland security (HS)-related applications by developing test plans, conducting tests, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with quality assurance (QA) protocols to ensure that data of known and high quality are generated.

In the event of a large urban release of *B. anthracis* spores, extensive resources would be required in the recovery effort, and the number of private decontamination contractors available may not be sufficient to respond to the decontamination needs (Krauter and Tucker 2011). Chlorine dioxide (ClO_2) fumigation was used to decontaminate four buildings following the Amerithrax incident (Canter 2005), and has been identified as an option for the decontamination of large indoor civilian areas following an aerosol release of *B. anthracis* spores over a wide urban area (Campbell et al. 2012). But because ClO_2 gas concentrations at 10% or above tend to be unstable, and attempts to store the gas in compressed form have been unsuccessful, it must be generated at the point of use (Wood and Martin 2009).

A primary factor affecting the feasibility of using ClO_2 fumigation to successfully decontaminate a large building contaminated with *B. anthracis* spores is the target ClO_2 concentration. The ClO_2 generation technology must be capable of producing ClO_2 at a high enough rate to achieve the relatively high target concentration in the structure within a reasonable amount of time, while overcoming the loss of gaseous ClO_2 due to building air leakage and withdrawal, material demand, and chemical decomposition (US EPA 2008). For example, it was determined in a field study that a ClO_2 generation rate of ~ 54 kg/hr would be needed to achieve 1,500 ppm ClO_2 in a 9,900 m^3 building (Wood and Martin 2009). With this in mind, there may be only a few companies in the US with the technology capable of generating ClO_2 at such high rates.

Historically, ClO_2 fumigation for decontamination of buildings contaminated with *B. anthracis* spores has been performed with relatively high levels of ClO_2. Following the Amerithrax incident, three of the four anthrax contaminated buildings that were fumigated with ClO_2 were done so using a target ClO_2 concentration of 750 ppm, with a dwell time of 12 hours (US EPA 2005), while the fourth building (American Media Inc., located in FL) was fumigated at 3000 ppm for 3 hours (US EPA 2012 A). Fumigating with ClO_2 at 3000 ppm for 3 hours was also used at the full-scale Bio-Response Operational Testing and Evaluation (BOTE) field test (US EPA 2013). Further, many laboratory (bench-scale) studies reported in the peer reviewed literature on the sporicidal efficacy of ClO_2 gas have been conducted with ClO_2 at levels

typically around 1000 ppm or higher; see for example Rastogi et al. (2009); Jeng and Woodworth (1990); and Han et al. (2003).

In contrast, the study presented in this report focused primarily on using ClO_2 at relatively low concentrations but with longer contact times. Demonstration of successful decontamination efficacy with ClO_2 gas at concentrations lower than what has been used previously would allow for a greater number of vendors to participate in remediation efforts following a large anthrax spore release. That is, vendors with technologies that produce ClO_2, but perhaps at relatively lower generation rates, would still be able to meet the target fumigation concentration within a large building. Lower levels of ClO_2 gas would also presumably have less detrimental impacts on materials.

This investigation focused initially on proof of concept decontamination tests at bench scale using small coupons placed inside a small chamber (modified glovebox). Six tests were conducted, using target ClO_2 concentrations of either 100 or 200 ppm, with contact times ranging from 2-12 hours. The coupon materials used included wood, concrete, carpet, painted drywall paper, and galvanized metal.

These small scale tests were then followed by pilot-scale tests using a mock office set-up in a large decontamination chamber. The majority of the mock office tests used relatively low levels of ClO_2 gas (100-300 ppm), although a few tests were conducted at 750 ppm or higher to allow for comparison of results from previous studies. The mock office tests involved extensive surface sampling and other microbiological assays. A portion of the mock office tests included the use of computers to assess the impact of low level ClO_2 fumigation on sensitive electronics. Some of the mock office tests (during the latter portion of the study) also included the use of small coupons of different materials, to compare decontamination efficacy results between the coupons and surfaces within the office.

Decontamination efficacy tests were conducted with spores of *Bacillus subtilis* in the small scale tests, and with *B. atrophaeus* (a variant of *B. subtilis*) in the pilot-scale tests. Both microorganisms have been demonstrated as appropriate surrogates for *B. anthracis* in decontamination studies using ClO_2 gas (US EPA 2012B; US EPA 2013).

2.0 Summary of Test Procedures

2.1 Small-Scale Chamber Tests

Decontamination tests with ClO_2 gas were first conducted at small scale in an opaque exposure chamber (317 L) (Model 830-ABC, Plas-Labs, Inc., Lansing, MI), modified to maintain and control a leak-free fumigation atmosphere inside the exposure chamber, and to allow for the periodic addition and removal of coupons during fumigation.

Chlorine dioxide was generated using a ClorDiSys GMP ClO_2 generator and first routed to a mixing chamber to maintain a ClO_2 level at 750 ppm. When the concentration in the exposure chamber was below the set-point (e.g., 100 or 200 ppm), a solenoid valve directed the 750 ppm ClO_2 gas from the mixing chamber into the exposure chamber. Relative humidity (RH) inside the exposure chamber was controlled by a custom-built data acquisition system (DAS), using a Vaisala RH/temperature sensor (Model HMD40Y; Helsinki, Finland) to provide a signal used in a feedback loop. Humid air was supplied as needed using a gas humidity bottle (Fuel Cell Technologies, Inc. Model LF-HBA, Albuquerque, NM) heated to 140 °F. Temperature was controlled if necessary by circulation of cooling water through radiators located within the exposure chamber. A fan was used inside the exposure chamber to provide internal mixing. Additional details on the ClO_2 generator and measurements, and temperature and RH measurements, are presented below in Section 2.2 as well as in another reference (Wood et al. 2010).

Spores of *Bacillus subtilis* were used in the small scale tests as a surrogate for *B. anthracis* (US EPA 2012B), and were applied to small coupons (18 mm diameter) as an aerosol using a metered dose inhaler (MDI) provided by the Edgewood Chemical and Biological Center. The coupons were made from wood, concrete, carpet, painted drywall paper, and galvanized metal. Further details on the spores, the MDI deposition method, coupons, and the spore extraction and counting methods are described elsewhere (Lee et al. 2011). The target dose for each coupon was 10^7 to 10^8 colony forming units (CFU).

Six small-scale fumigation tests were conducted as follows: (1) place the appropriate coupons for the test in the exposure chamber and seal the chamber airlock; (2) establish the target temperature (24 ± 2°C) and RH (75± 5 %); (3) charge the chamber with ClO_2 to achieve the target concentration (either 100 or 200 ppm ± 10 % of target value); (4) maintain the target concentration, temperature, and RH for the specified time (note: time zero is defined as the time at which the target concentration was achieved in the chamber); (5) aerate the chamber for a defined length of time and until a safe ClO_2 concentration is achieved in the chamber; and (6) open the chamber and remove the coupons, placing them in the appropriate sample packaging containers. Coupons were also removed through the airlock (without affecting the chamber conditions) at intermittent time points in the fumigation cycle.

One Petri dish was used to contain a stainless steel stage holding a negative (blank) coupon and five replicate coupons for each material and time point. The Petri dish was transferred to the test chamber, and the cover was removed to allow exposure. Once a given time point was reached, the dish was transferred out of the chamber using the air lock. The decontaminated coupons

were then returned to the Biocontaminant Laboratory for analysis as soon as possible. Test coupon CFU quantification and decontamination efficacy were determined as described by Meyer et al. (2013). Briefly, efficacy for the small coupon tests is reported in terms of $\log_{10}$ reduction (LR), and was calculated as the mean of the $\log_{10}$ CFU counts for the control coupons minus the mean of the $\log_{10}$ CFU counts for the test coupons.

2.2 Mock Office Procedures

2.2.1 Biological Agent and Dissemination

The test organism for the mock office study was *Bacillus atrophaeus* (American Type Culture Collection (ATCC) 9372), in a dry spore form mixed with silicon dioxide particles (e.g., as a powder). *B. atrophaeus* was formerly known as *B. subtilis* var *niger* and also *Bacillus globigii*, and was used as a surrogate for *B. anthracis* in all three decontamination test rounds (including ClO_2 fumigation) in Phase 1 of the BOTE study (US EPA 2013). The bacterial spores were obtained from the U.S. Army Dugway Proving Grounds, and prepared by Dugway as reported in Brown et al. (2007A).

For each experiment, spores were released into the large decontamination chamber using ~ 0.2 g of the preparation, or approximately 2.0×10^{10} CFU. Ten L/min of clean, dry air was supplied to a fluidized bed aerosol generator (TSI Inc., Model 3400A, Shoreview, MN) loaded with the spore preparation, and run for 30-60 minutes. An Electrical Low Pressure Impactor (Model ELPI, Dekati, Ltd., Tampere, Finland) was used to monitor aerosol levels in the chamber in real time, to ensure proper functionality of the aerosol generator, bacterial spore release, and the deposition process. The fluidized bed was placed on a small table 1.27 m off the floor in the chamber, between two large mixing fans, and a perforated diffusion shield was placed over the fluidized bed to ensure uniform aerosol dissemination. Following release, the spores were allowed to settle overnight onto mock office surfaces within the decontamination chamber.

2.2.2 Decontamination chamber and test environment

All tests were conducted in the COnsequence MANagement and Decontamination Evaluation Room, or COMMANDER. COMMANDER consists of a stainless steel-lined inner chamber built specifically for decontamination testing, with internal dimensions of approximately 3.4 m wide, 2.5 m deep, and 2.8 m high. At the entrance to the chamber is an airlock compartment, and enclosing the chamber and airlock is an exterior steel shell. All three components are kept under cascading slight negative pressure (most negative within the inner chamber) with the use of separate air streams with valve controls on the inlet and outlet of each. Air entering the decontamination chamber passes through a high efficiency particulate air (HEPA) filter, and exhaust air from the decontamination chamber is ducted to an activated carbon bed and HEPA filter prior to release to the facility exhaust system. Fans were used inside the chamber to provide internal mixing during fumigation. The inner chamber inlet and outlet duct fans were turned off during fumigation, and the inlet duct valve was closed, but the outlet valve was left open to maintain a negative pressure inside the chamber. Further details and a diagram of COMMANDER can be found elsewhere (Wood et al. 2013).

Within the chamber, temperature, RH, air pressures, flow rates, and air duct valves are controlled and/or their data logged continuously using a supervisory control and data acquisition (SCADA) system. The RH and temperature within the decontamination chamber were measured with a

temperature and RH transmitter (Vaisala Inc., Model HMD40Y; Helsinki, Finland). This instrument was calibrated prior to each test by comparing its RH data with known RH values generated in the sealed headspace above individual saturated solutions of various salt compounds. The RH meters were replaced if calibration criteria could not be met. Temperature within the chamber was monitored but not controlled, and varied from 25-30 °C. The target RH for all tests was 75%; the chamber RH was increased as needed through the use of a custom-fabricated steam injection unit.

The test chamber facility is equipped with a vaporous hydrogen peroxide (VHP®) generator (STERIS Corporation, Model 1000 ED, Mentor, OH), and was used to sterilize (i.e., reset) the chamber and airlock prior to each test. The reset fumigation was typically conducted using a hydrogen peroxide (H_2O_2) concentration of 250-400 ppm for 4-6 hours. Hydrogen peroxide levels within the chamber were measured with an electrochemical sensor (Model B12, Analytical Technology Inc., Collegeville, PA, USA). This sensor is connected to the SCADA and used to verify that sterilization cycle conditions were met, and that after chamber aeration, levels are low enough for reentry.

2.2.3 Mock office description

The stainless steel surfaces of the decontamination chamber were covered by materials typical of an indoor office setting. The floor was covered with plywood and then industrial carpet tiles (Beaulieu Solutions, P/N 6579-8586-1200-AB). The rear and side walls were framed and faced with 1.27 cm thick drywall (GoldBond, P/N GB4080-0800). The drywall was patched with joint compound (USG Sheetrock, P/N 380119048) and joint tape (USG Sheetrock, P/N 382199010) according to typical building practices, then primed (Kilz, P/N 20005) and painted (Behr, P/N 105001). At the top of the walls, a drop ceiling was installed and consisted of acoustic ceiling tile panels (Armstrong, P/N SC1135c) and two plenum grilles to enable conditioning of the interior chamber air using the existing RH and temperature controls. The chamber was furnished with office equipment consisting of a laminated desk, an office chair, a file cabinet, pin cushion screen, books/catalogues, and a computer with monitor and keyboard. See Figure 2-1 for a photograph of a corner of the office, showing furniture and sample grid markings.

2.2.4 ClO_2 generation and measurement methods

Chlorine dioxide was generated using a Model GMP (ClorDiSys, Lebanon, NJ). The generator passes 2 percent chlorine gas in nitrogen through cartridges containing solid sodium chlorite and other proprietary ingredients. The system includes a photometric detector for continuous measurement (a data point collected every 10 sec) of ClO_2, and a feedback loop is used to maintain the chamber at the set-point concentration. The generator was located inside the airlock during operation but had a gas sampling line to withdraw air samples from the center of the interior chamber. Chlorine dioxide levels within the chamber as measured by the generator were verified using a wet chemistry method, with gas samples collected every 120 min and routed to impingers containing a solution of potassium iodide phosphate buffer. This impinger liquid was then analyzed for ClO_2 via titration with sodium thiosulfate. Additional details on the ClO_2 generation and measurement methods are described elsewhere (Wood et al. 2010).

Figure 2-1. Photograph of Portion of Mock Office Showing Furniture and Sample Grids

2.2.5 Test sequence

The general sequence of events for each test was as follows:

1. Target surfaces and equipment were arranged in the chamber as needed and the chamber airlock was sealed.
2. The decontamination chamber was reset using a hydrogen peroxide vapor decontamination cycle, then aerated.
3. The chamber was entered and surfaces sampled to determine background levels of any residual contamination; these samples are referred to as field blanks (FB) or "reset" levels. (Bacterial spore sampling and analytical methods discussed below.)
4. *B. atrophaeus* spores were then disseminated into the chamber/mock office using the fluidized bed aerosol deposition technique and allowed to settle overnight.
5. The chamber was entered a second time and surfaces sampled to determine the initial levels of spores (positive controls).
6. Temperature monitoring began and 75% RH was established within the chamber.
7. The chamber was charged with ClO_2 to achieve the target concentration.

8. The target concentration and RH were maintained for the specified contact time (Note: Time zero is defined as the time at which the target concentration was achieved in the chamber.).
9. The chamber was aerated until ClO_2 concentration was below 0.5 ppm in the chamber.
10. The chamber was entered a third time and sampled after fumigation (test samples).

2.2.6 Bacterial spore sampling and analysis

Numerous microbiological samples and assays were used to characterize bacterial spore presence or absence in the mock office for each experiment (115 total for each test). Samples were collected following the reset, dissemination of spores, and post-decontamination with ClO_2. Samples or assays were either quantitative (providing a numerical result) or qualitative (indicating either presence or absence of bacterial growth). The use of material coupons began about halfway through the study. Laboratory blanks of items such as growth media and sampling materials were also employed in each experiment to check for aseptic conditions. A summary of the number and type of samples/assays for each experiment is shown in Table 2-1. Each sample or assay is further described in the narrative below.

Table 2-1. Summary of Sample Methods and Assays

Sample or Assay type	# samples post reset (field blanks)	# samples post dissemination of spores (positive controls)	# samples post decontamination
Surface sampling with wipes or "vacuum socks"	10	25	25
Surface sampling with swabs		1	3
Air samples	1	1	1
Reference measurement coupons		6	1
Biological indicators		3	5
Material coupons	3	12	18
Total	14	48	53

Bacterial spore samples were collected from surfaces with methods based on the use of wipes, swabs, or hand-held vacuums equipped with a collection sock (referred to as "vacuum sock"). The wipe and vacuum sock samples were extracted and the recovered viable spores were quantified, while the swab samples were analyzed qualitatively for growth. A summary of the surfaces sampled within the mock office and method used is listed in Table 2-2 below. A total of 25 quantitative surface samples were collected in the office to characterize the spore loading (positive controls), and a total of 25 quantitative samples taken after ClO_2 decontamination (test samples). A total of 10 field blank samples were collected for each test as well (one wipe or vacuum sock sample for each surface). The selection of sampling method for each surface type was based on previous spore sampling studies (Brown et al. 2007A; Brown et al. 2007B; Brown et al 2007C.

Table 2-2. Sampled Material Surfaces for Mock Office Configuration

Material Surface	Sample technique	Surface area per sample (cm^2)	Number of positive controls and post-decon samples per test	Number of field blanks
Keyboard	Vacuum sock	564	1	1
Carpet	Vacuum sock	929	5	1
Ceiling Tile	Vacuum sock	929	3	1
Office Chair Seat	Vacuum sock	465	2	1
Office Chair back	Vacuum sock	465	2	1
Catalog	Vacuum sock	97	1	1
Pin cushion screen	Vacuum sock	929	3	1
Desk Table	Wipe	929	2	1
Filing cabinet	Wipe	1022	1	1
Painted Drywall	Wipe	929	5	1
Electrical socket faceplate	Swab	NA	1	0
Computer chassis	Swab	NA	1*	0
Keyboard	Swab	NA	1*	0

*The computer chassis and keyboard were swab sampled only following decontamination; NA = not applicable

The sampling locations for the wall, table, ceiling, and floor were determined using a grid approach (e.g., sample template), similar to the method as described in Wood et al. (2013). Smaller or larger size templates were used for the sampling of other surfaces to ensure a consistent sample size. Wipe samples were collected using *c.* 26 cm^2 gauze wipes (Kendall # 8402; Covidien, Inc., Mansfield, MA, USA); further details on the wipe pattern, wetting agent, and procedures for extraction of spores from the wipes can be found elsewhere (Calfee et al. 2012). Vacuum sock sampling (sock filtration kit (Midwest Filtration, p/n FAB-20-01-001A; Model Omega, Atrix International, Inc., Burnsville, MN) and methods used to extract spores from vacuum socks followed protocols outlined by Brown et al. (2007C).

Following spore extraction procedures, the liquid solution used to extract spores from each wipe or vacuum sock sample was 10-fold serially diluted and plated. When fewer than 30 CFU were detected on plates from undiluted extract, a portion of the remaining extraction solution was filter plated using one or two larger volumes of the extract. Plates were incubated for 18-24 hours at 35 ± 2 °C, and then colonies were counted. Further details on these laboratory analysis and plating techniques are described elsewhere (Calfee et al. 2012).

Total recovery (CFU quantity) for a surface sample was then calculated using equation 2-1. Briefly, the average number of colonies for the triplicate plates is multiplied by the reciprocal of the dilution (for which CFU counts that ranged between 30-300); then dividing by the plated volume (0.1 mL for standard plating, or the volume(s) of extraction liquid that was filter plated,

typically between 1-9 mL); and then multiplying by the total volume of the liquid used to extract spores from wipe or sock sample (typically 10 mL). The CFU loading for a particular sample was adjusted for the surface area sampled to arrive at a CFU count per square foot. When a filter plate for a particular post-decontamination (or blank) sample had no CFU detected, a CFU value of 0.5 CFU count was assigned to the filter. As an example, if no spores were detected on a filter from a sample in which 4 mL (out of 10 mL total extraction liquid) were filter plated, the sample itself would be assigned a value of 1.25 CFU.

$$\text{CFU/unit area} = (C/I)*D*V/A \qquad (2\text{-}1)$$

Where:

C = Counts per plate;

I = Volume of solution plated (mL);

D = Dilution factor (e.g., 1000 for a 1000:1 dilution, or 1 for filter plate);

V = Volume of extraction solution (mL): and

A = Area sampled

One swab sample was collected from the electrical socket faceplate following spore dissemination (prior to decontamination), as a positive control. Three swab samples were collected in the office following decontamination: the electrical socket face plate, computer chassis, and keyboard. Swab samples were used due to the complexity of the surface and were collected following protocols by the US Centers for Disease Control and Prevention (2012) using Bactiswabs™ (Remel, Lenexa, KS). The swab samples were used to evaluate only the presence or absence of spores before and after decontamination. Following the collection of sample, each swab was then streak plated on a TSA plate and incubated at a 35 °C ± 2 °C for at least 18 hours and checked for growth of the target organism.

Reference material coupons (RMCs) were also used to determine spore deposition, but without the need for surface sampling. The RMCs were 50 mm x 25 mm pre-sterilized rectangles of stainless steel placed throughout the chamber, with spores recovered from the RMCs directly by extraction in 50 mL conical vials (to avoid any bias from sampling methods). The RMCs were placed at six locations within the office: on the keyboard, in the center of the chair, on top of the filing cabinet, in the corner of the floor, and on a wall. Two RMCs were placed inside the computer chassis, with one recovered before and one RMC recovered after fumigation. The RMCs have a dog ear, which points up on the outward- facing surface, to allow for aseptic pick up. RMC CFU loadings were quantified using the same techniques as described for the small coupons in Section 2.1.

The presence of aerosolized spores in the chamber was measured using a ViaCell (Zephon, Ocala, FL, USA) bioaerosol collection cassette, sampling at a rate of 15 L/min. An air sample was taken during surface sampling operations. Air sampling typically lasted for two hours; air samples were withdrawn near the center of the mock office. Further details on this method and analysis are found elsewhere (Calfee et al. 2012). Results for this type of sample are presented in CFU/m^3 air.

BIs were another assay used to assess effectiveness of the ClO_2 fumigation in inactivating bacterial spores. The BIs were comprised of nominally 10^6 spores of *B. atrophaeus* inoculated onto stainless steel discs, and wrapped in Tyvek envelopes. The BIs were obtained from Mesa Labs (Model 1-6100ST, Lakewood, CO) and placed inside the computer chassis, inside the filing cabinet, on the pincushion screen, on the carpet floor, and on the desk table following positive control sampling. The five test BIs were collected after fumigation and analyzed according to manufacturer instructions to determine whether any of the BIs exhibited growth of bacteria (survival of any spores). Three positive control BIs (not exposed to ClO_2) were also used in each test.

During the latter half of the study, the mock office tests included the use of small coupons so that results for the coupons could be compared to efficacy results based on surface sampling data within the office, as well as the small chamber coupon data. Coupons were made from ceiling tile, carpet, and painted wallboard paper. The 18 mm material coupons were inoculated with *B. atrophaeus* with MDIs using the methods described in Section 2.1 of this report. Two inoculum levels, roughly $1x10^4$ and 1×10^7 CFU per coupon, were used. One inoculated coupon per target inoculum level and one sterile coupon of each material type were placed in three different locations inside COMMANDER following positive control office sampling. Thus a total of 18 test coupons were used for each experiment (3 materials x 3 office locations x 2 inoculum levels). Two coupons, inoculated first and last, of each inoculum and material type, were not fumigated and served as positive controls. The CFU levels on the coupons were quantified as described in Section 2.1

2.2.7 Spore deposition and decontamination efficacy characterization

Spore deposition (i.e., pre-decontamination, positive control spore levels) was quantified by taking the logarithm_{10} of the CFU count for each wipe or vacuum sample (on a square foot basis), and then calculating the mean and standard deviation of the log values (the mean of a series of log values is equivalent to the log of the geometric mean for the same series) is for the pre-decontamination samples for each particular location. Post-decontamination results are presented in terms of spore recovery as well, and were calculated in the same manner as the pre-decontamination results. Results are also presented in terms of decontamination efficacy, which was quantified in terms of LR. The LR was calculated as the mean of the log values for each positive control sample location CFU count minus the mean of the log values for each test sample location CFU count. We occasionally report results by noting whether the average LR for a particular coupon or surface test is ≥ 6.0, since a decontaminant that achieves ≥ 6 LR is considered effective as a sporicidal decontaminant (US EPA 2010). We note, however, that while a decontamination efficacy ≥ 6 LR may be considered "effective" when reporting test results, in an actual *B. anthracis* release event, the goal for decontamination would be to minimize the number of recoverable spores, regardless of LR. Hence we also report results in terms of the number of samples in which spores were not detected, discussed next.

When a filter plate for a particular post-decontamination (or blank) sample had no CFU detected, the sample was scored qualitatively as "non-detect" (ND). The number of post-decontamination surface samples (out of 25 for a given COMMANDER test) that were found to have no spores detected is reported in the Results section. This approach also characterizes the effectiveness of the ClO_2 treatment, consistent with previous decontamination studies (US EPA 2013). When no spores were detected for a sample, this result implied the highest decontamination efficacy

quantifiable and achievable, and the LR was reported as ≥ the positive control recovery minus the recovery from the test sample (calculated based on imputing a 0.5 CFU value on the filter plate, and adjusting for the filter plate volume; see Sub-section 2.2.6).

2.2.8 Procedures for Assessing Impact of ClO_2 on Computer Operation

For five fumigation tests, three additional computers were installed in the mock office to assess the impact of low level ClO_2 fumigation on the functionality of the computers. The computer equipment used and tested included a Dell OptiPlex 780 Desktop Computer; a Dell 21.5 inch Flat Panel Monitor; a USB keyboard and mouse; and computer and monitor power cords and connecting analog video cable. Three computers were on and idle in each of the five tests. Three positive control computers (not exposed to ClO_2) were also evaluated for functionality.

Before and after fumigation, computer functionality was evaluated using a software diagnostic tool to determine the number of computer component failures. The post-fumigation analysis was conducted monthly for a period of one year following the fumigation date. During the one year period, all equipment was stored in an indoor office/laboratory environment with logged temperature and RH. A 5 day, 8-hour workweek was simulated by load-testing software (BurnIn Test, Passmark Software Pty Ltd.) for the computer systems between evaluations. Refer to Appendix B for further details on the methods used to assess computer functionality.

3.0 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.

3.1 Sampling, Monitoring, and Equipment Calibration

Approved operating procedures were used for the maintenance and calibration of all laboratory equipment. All equipment was verified as being certified calibrated or having the calibration validated by the EPA's Metrology Laboratory at the time of use. Standard laboratory equipment such as balances, pH meters, biological safety cabinets and incubators were routinely monitored for proper performance. Calibration of instruments was done at the frequency shown in Tables 3-1 and 3-2. Any deficiencies were noted. Any deficient instrument was adjusted to meet calibration tolerances and recalibrated within 24 hours. If tolerances were not met after recalibration, additional corrective action was taken, possibly including recalibration or/and replacement of the equipment.

Table 3-1. Sampling and Monitoring Equipment Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance
Meter box	Volume of gas is compared to NIST-traceable dry gas meter annually	± 2 %
Flow meter	Calibration using a flow hood and a Shortridge manometer	± 5 %
RH and temperature sensor	Compare RH to 3 calibration salts once a week; thermistor (for temperature) part of RH sensor and calibrated by manufacturer	± 5 %
Stopwatch	Compare against NIST Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days	± 1 min/30 days

Table 3-2. Analysis Equipment Calibration Frequency

Equipment	Calibration Frequency	Calibration Method	Responsible Party	Acceptance Criteria
Pipettes	Annually	Gravimetric	External Contractor	± 1 % target value
Incubator Thermometers	Annually	Compared to NIST-traceable thermometer	Metrology Laboratory	± 0.2 °C
Scale	Before each use	Compared to Class S weights	Laboratory staff	± 0.01 % target

3.2 Acceptance Criteria for Critical Measurements

QA/QC checks associated with this project were established in the QAPP. A summary of these checks is provided in Table 3-3.

Table 3-3. Summary of QA/QC Checks

Matrix	Measurement	QA/QC Check	Frequency	Acceptance Criteria	Corrective Action
ClO_2 measurement	Wet chemistry impinger method	Validation of generator reading	Once per hour	± 35 % of generator reading	Change generator cartridges if necessary
Negative Test Coupon Samples (swabs, wipe, or vacuum sock)	CFU	Field Blank	One per sample type per sampling event	0 CFU	Revise handling procedures; investigate for sources of contamination
Negative Material Coupon Samples (swabs, wipe, or vacuum sock)	CFU	Biocontaminant Material Blanks of PBST*, dilution tubes, and plating beads (check that plating materials are not contaminated)	3 per each material used, daily	0 CFU	Investigate sterilization procedure; investigate sources of contamination
Positive Test Coupon Samples (swabs, wipe, or vacuum sock)	CFU	Positive Controls (inoculated w/ spores, but not subject to any treatment)	Up to 5 per material per test	5E6 to 5E7 CFU	Assess deposition or sampling protocol
Test Coupon Samples (wipe or vacuum sock)	CFU	Agreement of triplicate plates of single coupon at each dilution	Each sample	Each CFU count must be within 100 % of the other two replicates	Replate or filter samples
Chamber RH	RH	2-point calibration	Once per day	± 5 %	Replace Vaisala sensor
Control Computers	Computer functionality per PC-Doctor®	Act as a baseline for comparison of fumigated computers	In triplicate, monthly	NA	NA

*Phosphate Buffered Saline with Tween® 20.

3.3 Data Quality

Temperature and RH measurement devices were maintained within the calibration tolerances found in Table 3-1. Fumigant concentrations were sampled using impingers and the impinger liquid was analyzed using wet chemistry techniques. These values were all within the acceptance criterion ±35 % of ClO_2 generator readings.

Designated surfaces were sampled prior to biological exposure to test the effectiveness of the reset. Figure 3-1 shows the total number of occurrences each sample surface/location showed *B. atrophaeus* CFU or non-target contamination prior to spore dissemination (after reset) for the entire test matrix of 13 tests. (Non-target contamination, when present, was typically white, glossy colonies easily distinguishable from the orange colored *B. atrophaeus* colonies.) With the exception of the filing cabinet and pin cushion, all test surfaces had a non-zero negative sample at least once during the project. Refer to Appendix A for further information regarding which surface(s) for each test were found to have target spores present following the reset.

For the majority of tests there was either no contamination found or only one surface was found to have target spores present following the reset. The maximum number of surfaces that were positive following the reset was four, which occurred in Test 11. In most cases in which surfaces were positive for spores following the reset, the actual number of spores detected per sample was less than 5 CFU using filter plating techniques.

Although the intention was to minimize the presence of contamination from the field blanks after reset, the levels are considered minor and not expected to impact results of the study. Spores present in the sampling field after sterilization could be indicative of cross-contamination during sampling, confounding post-fumigation results of the same order of magnitude. However, most likely they could be representative of an inadequate sterilization cycle. Once we switched to a more robust reset cycle (400 ppm for 6 hours) for use in the latter half of the study, contamination generally decreased.

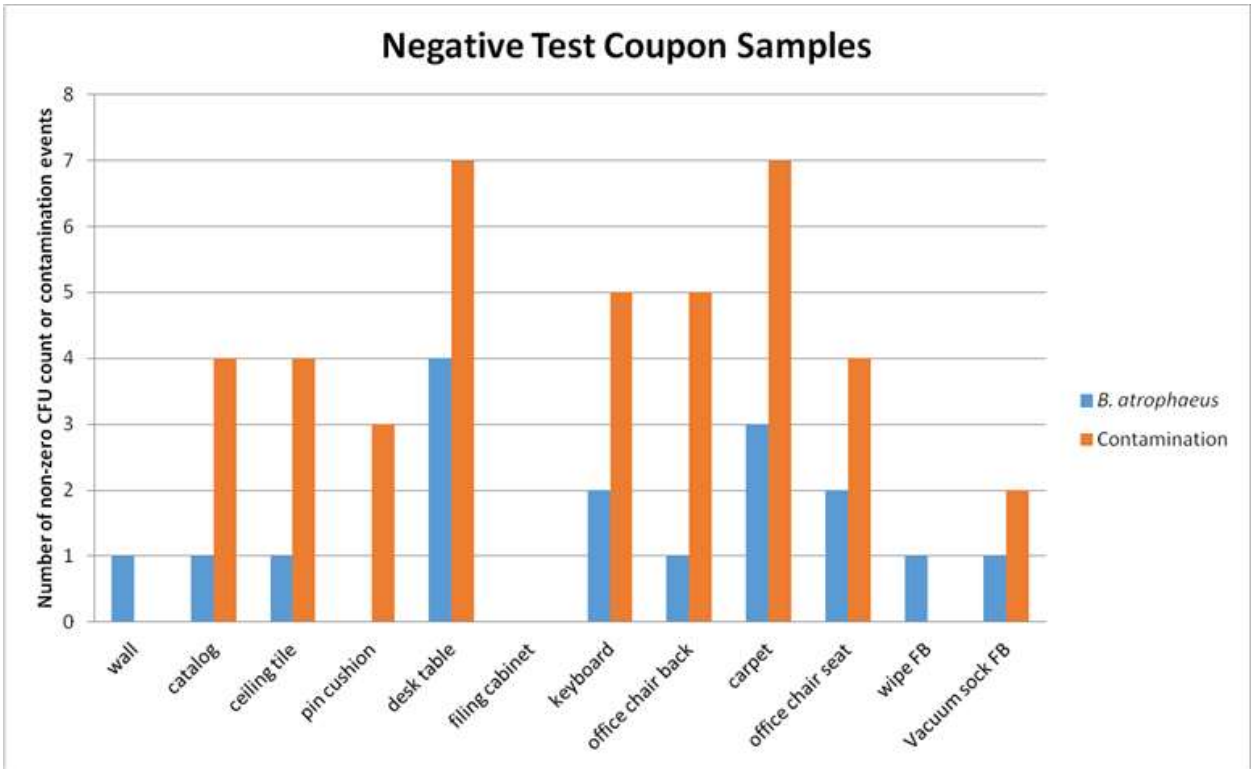

Figure 3-1. Field Blank Test Sample Results by Surface Sample

4.0 Results and Discussion

4.1 Small Scale Coupon Decontamination Results

A summary of the results (in terms of LR) for the small scale coupon tests is shown in Table 4-1. Contact times for the fumigations ranged from 2 to 12 hours.

Fumigation with ClO_2 was effective (> 6 LR) for nearly every condition tested except for the wood coupons at 200 ppm (all contact times), and galvanized metal at 100 ppm, 2 hours. (For wood coupons at the 200 ppm condition, average surface loadings of detectable viable spores were reduced by a minimum of 5 log CFU as a result of the decontamination process.) In the majority of the small coupon tests, spore loadings were reduced by more than 7 log. For example, in every test with concrete and painted drywall paper, the spores were completely inactivated, while for wood there were no tests in which the spore population was completely inactivated.

We note that in some cases, a higher dosage of ClO_2 resulted in an ostensibly lower LR result for the same material. For example, the LR for wood at 100 ppm, 8 hours (800 ppm-hour dose) was 6.61, while the LR at 200 ppm for 6 hours (1,200 ppm-hour dose) was 5.23. While appearing to have a higher LR at a lower dose, the results are not significantly different (MS Excel t-test). For this case, the lack of statistical significance may be due to the relatively variable results obtained for wood (compare standard deviations of efficacy results among the materials in Table 4-1), which in turn is most likely due to the complex, porous nature of this material affecting spore recovery. (However, one other explanation for the apparent difference in results for the wood coupons at the two different concentrations may be due to the complex (non-linear) inactivation kinetics (Ryan et al. 2014)). In particular, it was noted that when fumigating with ClO_2, the time required to achieve successful fumigation was determined to be independent of the fumigant concentration (Rastogi et al. 2010).

These small scale coupon test results suggest that low levels of ClO_2 gas may be a viable option for the decontamination of materials contaminated with anthrax spores, given there is sufficient contact time, RH and temperature. In many of the tests, relatively high efficacy was achieved at the first time point tested (2 or 4 hours), masking any potential effect of contact time. Of the materials tested, wood was the most difficult to decontaminate. Based on these proof of concept results, the study moved forward with the large scale office testing in COMMANDER.

Table 4-1. Summary of Decontamination Efficacy Results for Small Chamber Tests*

ClO_2 level and contact time (hr)	Wood	Concrete	Painted drywall paper	Industrial carpet	Galvanized metal
100 ppm					
2 hr					5.71 ±0.30
4 hr					7.49 ±0.39
6 hr					6.76 ±0.74
8 hr	6.61±0.90	> 7.67 ±0.23	> 7.88±0.29	7.72±0.51	6.33± 0.33
10 hr	6.67± 1.45	>7.68 ±0.23	>7.88±0.29	7.77±0.16	
12 hr	7.14 ±1.10	>7.68± 0.23	> 7.88±0.29	7.67±0.47	
200 ppm					
2 hr					7.54 ±0.47
4 hr	5.4±1.51		> 7.69±0.48	> 7.76± 0.42	> 7.81± 0.12
6 hr	5.23±0.95	> 7.66±0.36	> 7.71±0.48	7.54± 0.51	> 7.81± 0.12
8 hr		> 7.60±0.36	> 7.71±0.48	> 7.78± 0.42	7.66 ±0.35
10 hr	5.35±0.92	> 7.59±0.36			

* Results reported as average LR ± SD; results presented as > indicate complete inactivation of spores (no spores detected). Blank table cells indicate material not tested at that condition

4.2 Office Decontamination Results

4.2.1 Test Matrix Summary and Fumigation Conditions for Mock Office Tests

A summary of the fumigation conditions for each mock office decontamination test is shown in Table 4-2. These values include the target and actual levels for ClO_2 concentration, contact time, RH, and temperature. Except as noted in the table, all fumigations had a target RH of 75 % and a target temperature of 25 °C. Also shown in the table is the charge time, which is the time required to achieve the target concentration within the chamber.

The RH was generally well controlled, and with the exception of one test, ranged from 74-78 %. In Test 15, a malfunction occurred with the humidification control system, and resulted in an average RH level of 88%. Actual average temperatures for the COMMANDER tests ranged from 22-29 °C.

Target ClO_2 levels ranged from 100 – 3,000 ppm, with contact times ranging from 3-24 hours. Actual ClO_2 levels were generally within ± 10% of target values, with the exception of some of the initial tests (e.g., Test 5). In addition, we were unable to achieve 3,000 ppm when this was used as a target level (Tests 15 and 18), possibly because the ClO_2 generator had inadequate

generation capacity to overcome the large chamber volume and material demand from the office materials (an issue discussed in Section 1 of this report).

Table 4-2. Summary of Fumigation Conditions for Mock Office Tests

Test #	Target conc. and contact time	Actual mean ClO_2 level (ppm)	Actual contact time (hours)	Actual mean RH %	Actual mean T (°C)	ClO_2 charge time (min)
3	100 ppm, 8 hours	129± 19	8	74.7	28.8	4
5	100 ppm, 12 hours	166± 67	12.2	75.2	22.9	46.6
4	200 ppm, 4 hours	228± 18	4	75.7	29.0	22
6	200 ppm, 6 hours	214± 32	6	74.5	27.7	11
14B	200 ppm, 8 hours	215± 34	8	75.2	23.8	8
19	200 ppm, 8 hours	198± 26	8	76.7	21.5	10
8B	300 ppm, 4 hours	326± 19	4	76.6	29.9	30.5
16	300 ppm, 24 hours	327± 27	24.8	75.3	25.6	12.3
11	750 ppm, 12 hours	703± 157	12	75.7	25.2	41
13	1000 ppm, 9 hours	943± 59	9.2	78.2	23.8	80
17	1000 ppm, 12 hours	1021± 35	12	75.2	23.3	76
15	3000 ppm, 6 hours	956 ±310	7	87.8	28.7	49
18	3000 ppm, 3 hours	2567± 177	3	75.4	24.2	275

4.2.2 Quantitative surface and air sampling results for office

A summary of the overall decontamination results for the mock office tests is shown in Table 4-3. The results presented include average office spore levels before and after decontamination with ClO_2, in log CFU/ft^2, for each test. In addition to these quantitative results, decontamination effectiveness is characterized qualitatively in terms of the number of office surface samples (out of 25 per test) in which there were no spores detected after filter plating (designated as non-detect, or ND). Lastly, spore concentration in the air, before and after decontamination, is also shown in Table 4-3. Further details of these results, including the spore levels for each particular office surface, for each test, before and after decontamination, are found in Appendix A.

Table 4-3. Summary of Decontamination Results For Mock Office Tests

Test #	Actual Mean ClO_2 level (ppm)	Contact time (hours)	Avg. spore office loading log CFU per sq ft, prior to decontamination	Avg. spore loading, log CFU per sq ft, post decontamination	Number of post decon office samples that were ND (out of 25)	Number of post decon swab and RMC samples ND (out of 4)	CFU/m^3 air pre decon	CFU/m^3 air post decon
3	129± 19	8	5.68±0.59	1.43±1.21	9	4	2,590	9.65
5	166± 67	12	6.39± 0.75	2.24± 1.77	4	1	23,500	80
4	228± 18	4	5.35± 0.70	-0.01± 0.36	24	4	243	21.7
6	214± 32	6	4.89± 0.74	0.14± 0.46	22	4	3,860	5.69
14b	215± 34	8	7.01± 0.61	1.55± 0.78	9	4	19,800	62.5
19	198± 26	8	5.73 ± 0.72	0.17± 0.48	22	4	3,830	2.14
8b	326± 19	4	5.89± 0.63	0.62± 0.60	12	3	1,670	10.5
16	327± 27	24	6.63± 0.71	1.32± 1.25	14	3	60,000	29.5
11	703± 157	12	6.61± 0.58	0.66± 0.67	12	3	14,600	10.9
13	943± 59	9	7.21± 0.40	1.33± 0.65	16	2	37,900	43.4
17	1021± 35	12	6.58± 0.51	0.70± 0.84	19	3	21,900	1.44
15	956* ±310	6	7.01± 0.51	1.02 ±0.88	19	4	35,500	10.5
18	2567± 177	3	6.82± 0.56	1.21± 0.40	24	4	27,100	5.88

- *Test conducted at 85% RH

The average spore loadings for the mock office, prior to decontamination, ranged from 4.89 – 7.21 log CFU/ft^2. These levels were in proximity to our target range of 5-7 log CFU/ft^2. As expected, the spore loadings prior to decontamination for horizontal surfaces within the office (e.g., desk table, but excluding ceiling tiles) were approximately 0.5 log CFU/ft^2 higher than the vertical surfaces (e.g., office walls), while the ceiling tile levels were generally approximately 0.5 log CFU/ft^2 lower than the vertical surfaces.

Overall average spore loadings for the office following decontamination ranged from -0.01 to 2.24 log CFU/ft^2. (A negative log value implies a spore loading < 1.0 CFU/ft^2).

The most difficult office objects to decontaminate (had the highest spore loading following decontamination, or had the fewest samples that were non-detect for the study) were the horizontal surfaces (excluding the ceiling tiles). For example, there were only two tests in which no spores were detected on the laminated desktop following ClO_2 fumigation. Items such as the walls and ceiling, with the lowest pre-decontamination spore levels, had the highest number of tests in which no spores were detected on their surfaces post-decontamination.

In an effort to assess the importance of various fumigation and related parameters on overall decontamination efficacy, we conducted correlations (Pearson's r) of these data for each test using MS Excel. The results are presented in Table 4-4. The results of this analysis show a mild correlation of 0.62 between pre-and post-decontamination spore levels.

Table 4-4. Correlation of Fumigation Parameters and Decontamination Results

Parameter 1	Parameter 2	Correlation (r)
Pre-decontamination spore surface loading	Post-decontamination spore surface loading	0.62
Average ClO_2 concentration	LR	0.46
ClO_2 concentration * contact time	LR	0.64
Post-decontamination spore surface loading	Post-decontamination spore air levels	0.74
Pre-decontamination spore surface loading	Pre-decontamination spore air levels	0.69
Number of post-decontamination surface samples that were negative	LR	0.46
Pre-decontamination spore surface loading	Pre-decontamination spore surface loading on RMCs	0.90
Number of post-decontamination surface samples that were negative	Number of post-decontamination swab and RMC samples that were negative	0.56
Number of post-decontamination surface samples that were negative	Number of post-decontamination coupons (mock office tests) that were negative	0.56

For the overall study, Test 4, with only a 228 ppm ClO_2 average level and 4 hour contact time, resulted in the highest number of surface samples post-decontamination that were negative (24 out of 25, or 96 %). This test also had lowest quantity of recovered spores following decontamination, and also had the second lowest spore loading prior to decontamination. The average post-decontamination spore level for Test 4 was determined to be approximately 1 CFU/ft^2, calculated from the assumption that the ND samples had a level of 0.5 CFU on the filter plate.

The direct effect of spore loading on efficacy may also be viewed by comparison of the results for Tests 14B and 19, which had similar fumigation parameters (~ 200 ppm ClO_2 for 8 hours). The number of non-detects for Test 19 was much higher (22, compared to 9 for Test 14B), while the pre-decontamination spore loading for Test 19 was over 1 log CFU/ft^2 lower than for Test 14B.

Test 18, which had the highest overall average ClO_2 concentration (2,567 ppm), also resulted in 24 of 25 samples that were non-detect. However, the average spore loading following decontamination was 1.21 log CFU/ft^2 (or 16 CFU/ft^2). (This relatively mid-level spore loading following decontamination, associated with a high number of non-detects, is due to the variable detection limits.) Depending on the surface area sampled and the extraction liquid volume filter plated, a non-detect (on a filter plate, assumed to be 0.5 CFU; refer to Section 2.2.6) result could correspond to between 0.5 to 20 CFU/ft^2.

The result of decontaminating with a high level of ClO_2 for a short contact time is comparable to other studies that have generally shown high efficacy (or relatively low levels of CFU post-decontamination) with 3,000 ppm ClO_2, but not complete decontamination (i.e., not all samples

were non-detect). See for example US EPA (2013) and Rastogi et al. (2009). The comparability of results between the present study high ClO_2 test and these other tests provides some verification of the methods used in the present study.

Because of the variation in pre-ClO_2 spore loadings and other parameters (e.g., concentration) associated with conducting large-scale tests, it becomes difficult to directly assess the effect of these parameters on outcome. For this reason, the correlations were performed. As shown in Table 4-4, the product of ClO_2 concentration times contact time (dose) had the highest correlation ($r = 0.64$) with decontamination efficacy. The fumigation parameter ClO_2 concentration, by itself, had a correlation of 0.46 with LR. That concentration by itself had a lower correlation than dose was not unexpected, owing to the importance of time in microbial inactivation. Other typical fumigation parameters such as RH and temperature were not assessed for correlation, since these parameters remained fairly constant throughout the study.

While the results of the office tests are comparable to the small scale coupon tests presented in Section 4.1, it is difficult to make direct comparisons for the two scales of testing. Differences in materials, spore deposition methods, spore loadings, sampling procedures, surface sample size, etc., make direct comparisons difficult since these could affect efficacy results. For example, while nearly every LR result in the small scale tests was greater than 6, none of the mock office tests resulted in overall LR values of greater than 6; refer to Appendix A. However, there were several office tests in which a number of surfaces were decontaminated with LR > 6. For example, the filing cabinet and wallboard were decontaminated with LR greater than 6 in eight different tests.

One other possible source that could contribute to some of difference in results for the small and large scale tests is the resuspension of spore particles that occurred during sampling of the mock office. Post-decontamination sampling activities conducted in the confined office space may have reaerosolized any viable spores remaining on surfaces (such as carpet), due to tracking of personnel and movement of equipment. (Indeed, carpet was one of the more difficult materials to decontaminate in the office.) The results of the post-decontamination spore levels in the air are shown in Table 4-3, and range from approximately 1 to 80 CFU/m^3. Post-decontamination average spore surface levels are well correlated (0.74) with the spore levels in the air.

4.2.3 RMC and swab results

The average pre-decontamination spore loadings in the office, as determined by surface sampling, were highly correlated with pre-decontamination spore loadings on the RMCs. As noted in Table 4-4, the correlation for these two parameters was 0.90. The average RMC spore levels for a particular test were generally higher than the average loading determined by surface sampling, in terms of log CFU/ft^2. This result is consistent with the expected higher spore recoveries obtained through direct extraction of materials versus the extraction of spores recovered from surface sampling matrices such as wipes or vacuum socks. Typical differences between RMC levels and surface sampling levels were less than 0.5 log CFU/ft^2. Refer to Appendix A for detailed RMC results for each test.

One swab sample was collected from the electrical outlet faceplate for each test (post-spore dissemination, i.e., prior to decontamination), and for every test, the result was positive for growth. Following decontamination, there were three office surfaces (faceplate, keyboard, and computer chassis) that were sampled with a swab for qualitative results. In addition, an RMC

was located inside a computer to assess the post decontamination result. The results for these samples, in terms of the number (out of four per test) that were completely inactivated after decontamination (e.g., no growth shown for swab samples; no spores detected from RMC), are also shown in Table 4-3. The number of post-decontamination swab or RMC samples that were negative had a 0.56 correlation coefficient with the number of post decontamination samples determined by surface sampling that were negative.

4.2.4 Coupon results

The decontamination results for the ceiling tile, carpet, and painted wallboard mock office coupon tests are summarized in Table 4-5, in terms of the number of coupons (out of 18) that were completely decontaminated (no detectable spores, i.e., "non-detect") during each test. These results are compared to the COMMANDER surface sampling results to provide context and have a correlation coefficient of 0.56.

In addition, of the three materials used for coupons in the mock office tests (carpet, wallboard paper, ceiling tile), the carpet material had the highest number of coupons that had detectable spores following decontamination. This result is consistent with the small chamber tests (Table 4-1), in which the carpet coupons were more difficult to decontaminate than the wallboard paper coupons. Please see Table A-14 in Appendix A for additional details on the results of these coupon tests.

Table 4-5. Decontamination Results for Coupons Placed in Mock Office

Test #	Actual Mean ClO_2 level (ppm)	Contact time (hours)	Number of post decon coupons that were non detect (out of 18)	Number of post decon office samples that were non detect (out of 25)
14b	215± 34	8	15	9
19	198± 26	8	17	22
16	327± 27	24	10	14
17	1021± 35	12	18	19
15	956 ±310	6	18	19
18	2567± 177	3	17	24

4.2.5 BI results

All of the positive control BIs (those not exposed to ClO_2) from every experiment tested positive for growth, as expected. None of the BIs exposed to ClO_2 in any of the mock office tests (five BIs were used per test) exhibited growth. While inactivating all of the BIs in the whole experimental program demonstrates the general concept and utility of using low levels of ClO_2, many of the actual surfaces within the office still contained viable spores. This general result is

consistent with other tests (Rastogi et al. 2010) that have shown that the spore populations on BIs are typically much easier to inactivate than spores associated with coupons from building materials or actual environmental surfaces.

4.3 Impacts of Low level ClO_2 fumigation on computer operation

A summary of the results for assessing the impacts of ClO_2 fumigation on computer functionality over the period of a year is presented in Table 4-6. The results are presented in terms of the number of component failures, for both the test and control computers. Some of these failures were present before exposure to any ClO_2 fumigation, and so can be ruled out as an effect of fumigation. Table 4-6 shows the results with pre-existing component failure results removed.

Fumigation at approximately 214 and 211 ppm ClO_2 for 6 or 8 hours (Tests 6 and 7, respectively) and at approximately 303 ppm ClO_2 for 4 hours (Test 8) showed no measureable change on the function of the computers. The effects of the 12 hour, 166 ppm ClO_2 (Test 5) and the 8 hour, 297 ppm ClO_2 (Test 9) fumigation are more difficult to interpret. In both those cases, at least one computer performed as well as or better than the control computers, and at least one computer performed worse. See Appendix B for further details on the methods and results.

Table 4-6. Total Computer "Fail" Results over Year-Long Test Period

	No ClO_2 (control computers)	**214 ppm, 6.0 hr. (Test 6)**	**166 ppm, 12.2 hr. (Test 5)**	**211 ppm, 8.0 hr. (Test 7)**	**303 ppm, 4.3 hr. (Test 8)**	**297 ppm, 8.3 hr. (Test 9)**
Computer A	7	0	15	4	2	30
Computer B	6	0	36+42[a]	1	3	0
Computer C	0	0	7	3	1	0

a = This computer did not record the results of 42 tests for one test day

5.0 Summary and Conclusions

Extensive testing was conducted in this study to assess the feasibility of using relatively low levels of ClO_2 gas for decontamination of materials and volumetric spaces contaminated with *B. anthracis* spores. While levels of ClO_2 in the range of 1000-3000 ppm have previously been demonstrated to be mostly effective against *B. anthracis* spores, levels of ClO_2 on the order of 100-300 ppm (with accompanying longer contact times) have not been investigated. If ClO_2 fumigation at these lower concentrations were to be proven effective, vendors with technologies that produce ClO_2, but perhaps at relatively lower generation rates, would still be able to meet these lower target fumigation concentrations within a large building or other space needing to be decontaminated. This would allow for a greater number of vendors to participate in remediation efforts following a large anthrax spore release in an urban area.

To initially prove the concept, small chamber fumigation tests were conducted using 18 mm diameter coupons of wood, concrete, drywall, carpet, and galvanized metal, with spores deposited onto the coupons using MDIs. Several tests were conducted at either 100 or 200 ppm ClO_2, with contact times ranging from 2-12 hr, but with all fumigations conducted at room temperature and RH of 75 %. These small scale tests did indeed demonstrate conditions in which the low levels of ClO_2 were effective (> 6 LR) against the *B. anthracis* surrogate for all the materials tested.

After successful completion of the small scale tests, pilot-scale tests were conducted inside a mock office environment. To our knowledge, this portion of the study is the first of its kind, in terms of the number of tests conducted at near-full-scale, using ClO_2 fumigation. All tests were conducted with *B. atrophaeus* spores, a long-used surrogate for testing ClO_2 efficacy against *B. anthracis* spores. Spores were disseminated into the office as a dry powder to simulate a realistic release scenario. The study involved extensive surface sampling and other microbiological assays, employing sampling techniques that would be used in response to an actual anthrax incident. A portion of the mock office tests included the use of computers, to assess the impact of low level ClO_2 fumigation on sensitive electronics and functionality. While the focus of the study was to use relatively low levels of ClO_2 gas (100-300 ppm), a few tests were conducted at 750 ppm or higher to allow for comparison of results from previous studies. As with the small scale tests, all of the mock office fumigations had a target RH of 75 % and a target temperature of 25 °C.

The average office pre-decontamination spore levels ranged from 4.89 – 7.21 log CFU/ft^2, near our target of 5-7 log CFU/ft^2. Overall average spore loadings for the office following ClO_2 treatment ranged from -0.01 to 2.24 log CFU/ft^2, and generally correlated (0.62) with pre-decontamination spore loadings. The correlation was apparent for specific surfaces within the office, as well as for the overall average spore loadings in the office. For example, the most difficult office objects to decontaminate were the horizontal surfaces such as the desk table and carpet, which also tended to have the highest pre-decontamination spore loadings. Finally, post-decontamination spore levels in the office air ranged from approximately 1 to 80 CFU/m^3, and correlated (0.74) with the post-decontamination spore surface levels.

The mock office portion of the study demonstrated that relatively high decontamination efficacy (approximately 5 to > 6 LR) can be achieved using relatively low levels of ClO_2, depending on the pre-decontamination spore levels and other conditions. For example, Test 4, with only a 228 ppm ClO_2 average level and 4 hour contact time, resulted in the lowest quantity of spores

recovered following decontamination (calculated to be approximately 1 CFU/ft^2, based on assuming 0.5 CFU were recovered from ND samples). Correspondingly, this test also had the highest number of surface samples post-decontamination that were negative (24 out of 25, or 96 %) for the test program, and had the second lowest average office spore loading prior to decontamination.

Relative to comparing the results of the office tests to the initial small scale coupon tests, while nearly every LR result in the small scale tests was greater than 6, none of the mock office tests resulted in overall average office LR values of greater than 6. Nevertheless, there were several office tests in which a number of surfaces were ND for spores and were decontaminated with LR > 6. For example, the filing cabinet and wallboard were decontaminated with LR greater than 6 in eight of 13 different tests. In any event, it is difficult to make direct comparisons for the two scales of testing, since differences in materials, spore deposition methods, spore loadings, sampling procedures, surface sample size, etc., could affect efficacy results.

Every BI exposed to ClO_2 in all the mock office tests in which they were used was inactivated, demonstrating the general concept and utility of using low levels of ClO_2. However, many of the actual surfaces within the office still presented viable spores following decontamination. This general result is consistent with other tests that have shown that the spore populations on BIs are typically much easier to inactivate than spores associated with coupons from building materials or actual environmental surfaces. The general conclusion here is that BIs that remain viable after ClO_2 treatment would be a good indication that the ClO_2 fumigation failed. However, BIs that have been inactivated following ClO_2 treatment do not necessarily imply that the fumigation was successful.

Fumigation at approximately 200 ppm ClO_2 for 6 or 8 hours and at approximately 300 ppm ClO_2 for 4 hours showed no measureable change in the functionality of the computers.

The study has demonstrated the potential of using relatively low levels of ClO_2 gas, accompanied by longer contact times, for effective decontamination of surfaces and spaces contaminated by anthrax spores. However, this decontamination approach may be better suited for areas that are not heavily contaminated, i.e., that have spore loadings less than 5 log CFU/ft^2 and/or that do not contain significant quantities of porous materials such as carpet and wood. Further research is recommended to find efficacious low concentration conditions for heavily contaminated surfaces and for difficult to decontaminate materials.

6.0 References

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Appendix A

Detailed Microbiological and Decontamination Results

Table A-1. Test 3 Detailed Results

Office Surface or Object		ClO_2 PPM target = 100	Contact time =8	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		2.05E+05	1.66E+02	
keyboard		1.93E+06	1.27E+02	
chair back		4.69E+04	5.71E+02	
chair seat	G	5.48E+05	9.65E+01	
pin cushion screen		2.63E+05	8.09E-01	
ceiling tile		1.42E+05	2.27E+00	
carpet		2.59E+05	1.12E+02	
desk top	G	3.11E+06	8.10E+02	
drywall		7.67E+05	1.00E+00	ND
filing cabinet		2.48E+06	1.00E+00	ND
log of geo. mean; log reduction		**5.68**	**1.43**	**4.25**
Total # surface samples (out of 25) = ND				9
avg horiz surf (excl. ceiling tile)		1.42E+06	2.19E+02	
avg vert surf		3.59E+05	1.91E+02	
electrical socket swab		G	NG	
keyboard swab			NA	
computer swab			NG	
viacell air sample (CFU/liter)		2.59E+00	9.65E-03	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		6.68E+05		
inside computer		1.34E+06		
corner of floor		3.57E+04		
center of chair		1.46E+05		
keyboard		1.88E+06		
filing cabinet		1.10E+06		
inside computer post-test			ND	
log of geo mean RMC		**5.66**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

NA= not available. ND=not detected

Table A-2. Test 5 Detailed Results

Office Surface or Object		ClO_2 PPM target = 100	Contact time =12	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		**2.19E+05**	**6.00E+00**	**ND**
keyboard	**G**	**8.03E+06**	**7.90E+02**	
chair back		**1.08E+05**	**3.17E+02**	
chair seat		**3.93E+06**	**1.67E+03**	
pin cushion screen		**7.80E+06**	**9.06E+00**	
ceiling tile		**7.88E+05**	**1.45E+00**	
carpet		**2.97E+06**	**1.56E+03**	
desk top		**1.68E+07**	**1.06E+04**	
drywall		**2.59E+06**	**4.85E+01**	
filing cabinet		**1.35E+07**	**1.00E+03**	
log of geo. mean; log reduction		**6.39**	**2.24**	**4.15**
Total # surface samples (out of 25) = ND				4
avg horiz surf (excl. ceiling tile)		7.59E+06	2.61E+03	
avg vert surf		3.50E+06	1.25E+02	
electrical socket swab		**G**	**NG**	
keyboard swab			**G**	
computer swab			**G**	
viacell air sample (CFU/liter)		**2.35E+01**	**8.00E-02**	
viacell FB (CFU/sample)		**7.10E+00**		
Ref Measurement Coupons				
wall		**9.49E+06**		
inside computer		**3.90E+06**		
corner of floor		**5.03E+06**		
center of chair		**3.08E+06**		
keyboard		**1.12E+07**		
filing cabinet		**6.86E+06**		
inside computer post-test			**3.28E+03**	
log of geo mean RMC		**6.77**		

.

Note: all data in CFU/ ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-3. Test 4 Detailed Results

Office Surface or Object		ClO_2 PPM target = 200	Contact time =4	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		6.18E+04	6.00E+00	ND
keyboard		2.66E+06	9.40E-01	ND
chair back		3.55E+05	3.11E+00	
chair seat		1.73E+06	1.21E+00	ND
pin cushion screen		9.00E+04	6.32E-01	ND
ceiling tile		9.46E+04	6.12E-01	ND
carpet		2.54E+05	6.21E-01	ND
desk top		1.05E+06	5.81E-01	ND
drywall		7.60E+04	5.82E-01	ND
filing cabinet		1.97E+04	5.01E-01	ND
log of geo. mean; log reduction		**5.35**	**-0.01**	**5.36**
Total # surface samples (out of 25) = ND				24
avg horiz surf (excl. ceiling tile)		9.62E+05	1.64E+00	
avg vert surf		1.74E+05	1.44E+00	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		2.43E-01	2.17E-02	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		8.35E+03		
inside computer		2.88E+03		
corner of floor		1.44E+03		
center of chair		4.32E+03		
keyboard		1.15E+03		
filing cabinet		1.22E+07		
inside computer post-test			ND	
log of geo mean RMC		**4.05**		

.

Note: all data in CFU/ ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-4. Test 6 Detailed Results

Office Surface or Object		ClO_2 PPM target = 200	Contact time =6	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		5.76E+03	5.78E+00	ND
keyboard		1.53E+05	9.80E-01	ND
chair back		1.29E+04	1.21E+00	ND
chair seat	G	4.73E+05	1.21E+00	ND
pin cushion screen		2.57E+05	6.06E-01	ND
ceiling tile		8.61E+03	1.02E+00	ND
carpet	G	3.70E+04	7.36E-01	
desk top	G	4.26E+05	1.46E+01	
drywall		1.34E+05	6.56E-01	ND
filing cabinet		3.39E+05	6.27E-01	ND
log of geo. mean; log reduction		**4.89**	**0.14**	**4.76**
Total # surface samples (out of 25) = ND				22
avg horiz surf (excl. ceiling tile)		2.39E+05	3.99E+00	
avg vert surf		1.35E+05	8.24E-01	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		3.86E+00	5.69E-03	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		2.81E+05		
inside computer		1.56E+06		
corner of floor		1.26E+03		
center of chair		1.13E+05		
keyboard		3.13E+05		
filing cabinet		5.65E+05		
inside computer post-test			ND	
log of geo mean RMC		**5.17**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-5. Test 14B Detailed Results

Office Surface or Object		ClO_2 PPM target = 200	Contact time =8	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		1.34E+06	2.34E+01	
keyboard		3.97E+07	1.70E+02	
chair back		3.90E+06	2.00E+01	ND
chair seat		4.39E+06	7.00E+01	
pin cushion screen		1.00E+07	1.00E+01	ND
ceiling tile		1.86E+06	4.33E+01	
carpet		1.44E+07	2.87E+02	
desk top	G	9.68E+07	4.66E+02	
drywall		1.29E+07	1.80E+00	
filing cabinet		3.99E+07	5.15E+00	
log of geo. mean; log reduction		**7.01**	**1.55**	**5.46**
Total # surface samples (out of 25) = ND				9
avg horiz surf (excl. ceiling tile)		3.27E+07	1.70E+02	
avg vert surf		8.93E+06	1.06E+01	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		1.98E+01	6.25E-02	
viacell FB (CFU/sample)	ND			
Ref Measurement Coupons				
wall		2.09E+07		
inside computer		2.21E+07		
corner of floor		1.13E+07		
center of chair		1.08E+07		
keyboard		7.94E+07		
filing cabinet		4.12E+07		
inside computer post-test			ND	
log of geo mean RMC		**7.38**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-6. Test 8b Detailed Results

Office Surface or Object		ClO_2 PPM target = 300	Contact time =4	
	VHP ® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		1.32E+05	1.14E+01	
keyboard		2.90E+06	8.23E+00	
chair back		5.12E+04	5.23E+00	
chair seat		1.85E+06	1.26E+01	
pin cushion screen		1.35E+06	4.52E+00	
ceiling tile		4.00E+05	5.88E-01	ND
carpet	G	4.42E+05	1.84E+01	
desk top		4.56E+06	1.33E+01	
drywall		8.25E+05	6.44E-01	ND
filing cabinet		2.62E+06	6.18E-01	ND
log of geo. mean; log reduction		**5.89**	**0.62**	**5.27**
Total # surface samples (out of 25) = ND				12
avg horiz surf (excl. ceiling tile)		2.08E+06	1.08E+01	
avg vert surf		7.42E+05	3.47E+00	
electrical socket swab		G	G	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		1.67E+00	1.05E-02	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		1.70E+06		
inside computer		5.80E+06		
corner of floor		1.10E+06		
center of chair		1.93E+06		
keyboard		5.54E+06		
filing cabinet		2.68E+06		
inside computer post-test			ND	
log of geo mean RMC		**6.42**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-7. Test 16 Detailed Results

Office Surface or Object		ClO_2 PPM target = 300	Contact time =24	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		9.70E+05	5.82E+00	ND
keyboard		1.40E+06	1.48E+02	
chair back		2.40E+06	4.63E+00	
chair seat		8.17E+06	8.13E+00	
pin cushion screen		3.19E+06	6.06E-01	ND
ceiling tile		2.16E+05	3.74E+00	ND
carpet		6.23E+06	4.34E+02	
desk top		2.98E+07	1.12E+03	
drywall		1.27E+07	5.96E-01	ND
filing cabinet		4.78E+07	7.98E+02	
log of geo. mean; log reduction		**6.63**	**1.32**	**5.31**
Total # surface samples (out of 25) = ND				14
avg horiz surf (excl. ceiling tile)		1.57E+07	4.19E+02	
avg vert surf		6.10E+06	1.94E+00	
electrical socket swab		NA	NG	
keyboard swab			G	
computer swab			NG	
viacell air sample (CFU/liter)		6.01E+01	2.95E-02	
viacell FB (CFU/sample)		1.43E+01		
Ref Measurement Coupons				
wall		2.55E+06		
inside computer				
corner of floor		5.26E+06		
center of chair		**2.20E+07**		
keyboard		3.11E+07		
filing cabinet		1.16E+07		
inside computer post-test			ND	
log of geo mean RMC		**7.01**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-8. Test 11 Detailed Results

Office Surface or Object		ClO_2 PPM target = 750	Contact time =12	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		**5.82E+06**	**1.16E+01**	
keyboard		**3.86E+06**	**1.94E+01**	
chair back	**G**	**1.15E+06**	**2.32E+00**	
chair seat	**G**	**6.61E+06**	**2.91E+00**	
pin cushion screen		**2.64E+06**	**5.90E-01**	**ND**
ceiling tile		**3.30E+05**	**1.42E+00**	
carpet		**1.03E+07**	**2.22E+01**	
desk top	**G**	**2.74E+07**	**4.90E+01**	
drywall	**G**	**1.56E+06**	**6.30E-01**	**ND**
filing cabinet		**1.82E+07**	**4.64E+00**	
log of geo. mean; log reduction		**6.61**	**0.66**	**5.95**
Total # surface samples (out of 25) = ND				12
avg horiz surf (excl. ceiling tile)		1.20E+07	1.83E+01	
avg vert surf		1.79E+06	1.18E+00	
electrical socket swab		**G**	**G**	
keyboard swab			**NG**	
computer swab			**NG**	
viacell air sample (CFU/liter)		**1.46E+01**	**1.09E-02**	
viacell FB (CFU/sample)		**1.12E+00**		
Ref Measurement Coupons				
wall		**5.17E+06**		
inside computer		**3.24E+06**		
corner of floor		**2.13E+07**		
center of chair		**6.90E+06**		
keyboard		**5.85E+06**		
filing cabinet		**3.86E+06**		
inside computer post-test			**ND**	
log of geo mean RMC		**6.79**		

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-9. Test 13 Detailed Results

Office Surface or Object		ClO_2 PPM target = 1000	Contact time =9	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog	G	3.13E+06	1.44E+02	
keyboard		2.28E+07	3.29E+01	
chair back		9.52E+06	2.00E+01	ND
chair seat		3.60E+07	8.00E+01	
pin cushion screen		1.69E+07	1.00E+01	ND
ceiling tile	G	4.09E+06	1.00E+01	ND
carpet	G	3.17E+07	5.00E+01	
desk top		4.40E+07	5.43E+01	
drywall		1.63E+07	7.08E-01	ND
filing cabinet		3.22E+07	1.24E+01	
log of geo. mean; log reduction		**7.21**	**1.33**	**5.88**
Total # surface samples (out of 25) = ND				16
avg horiz surf (excl. ceiling tile)		2.83E+07	6.23E+01	
avg vert surf		1.42E+07	1.02E+01	
electrical socket swab		G	NG	
keyboard swab			G	
computer swab			NG	
viacell air sample (CFU/liter)		3.79E+01	4.34E-02	
viacell FB (CFU/sample)		1.73E+02		
Ref Measurement Coupons				
wall		2.44E+07		
inside computer		9.26E+06		
corner of floor		1.09E+07		
center of chair		4.70E+07		
keyboard		4.06E+07		
filing cabinet		3.04E+07		
inside computer post-test			3.65E+01	
log of geo mean RMC		**7.36**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-10. Test 17 Detailed Results

Office Surface or Object		ClO_2 PPM target = 1000	Contact time =12	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		3.60E+06	1.84E+02	
keyboard		1.05E+06	2.01E+00	
chair back		2.88E+06	1.22E+00	ND
chair seat		2.23E+06	1.22E+00	
pin cushion screen		2.18E+06	8.28E-01	
ceiling tile		1.00E+06	1.00E+01	ND
carpet		7.44E+06	1.00E+01	ND
desk top		3.65E+07	6.53E+01	
drywall		2.63E+06	5.62E-01	ND
filing cabinet		1.82E+07	6.18E+00	
log of geo. mean; log reduction		**6.58**	**0.70**	**5.88**
Total # surface samples (out of 25) = ND				19
avg horiz surf (excl. ceiling tile)		1.15E+07	4.47E+01	
avg vert surf		2.57E+06	8.70E-01	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		2.19E+01	1.44E-03	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		4.45E+07		
inside computer		7.69E+06		
corner of floor				
center of chair		1.32E+07		
keyboard		1.33E+07		
filing cabinet		1.91E+07		
inside computer post-test			7.83E+01	
log of geo mean RMC		**7.21**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-11. Test 15 Detailed Results

Office Surface or Object		ClO_2 PPM target = 1000	Contact time =6	**85% RH
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		4.30E+06	1.73E+02	
keyboard		3.88E+06	1.96E+00	
chair back		4.27E+06	2.00E+01	ND
chair seat		2.15E+07	2.00E+01	ND
pin cushion screen		9.55E+06	1.00E+01	ND
ceiling tile		1.98E+06	1.00E+01	ND
carpet		1.07E+07	1.60E+01	
desk top		8.37E+07	2.03E+02	
drywall		9.44E+06	6.82E-01	
filing cabinet		4.83E+07	5.15E-01	ND
log of geo. mean; log reduction		**7.01**	**1.02**	**5.99**
Total # surface samples (out of 25) = ND				19
avg horiz surf (excl. ceiling tile)		2.87E+07	6.91E+01	
avg vert surf		7.75E+06	1.02E+01	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		3.55E+01	1.05E-02	
viacell FB (CFU/sample)				
Ref Measurement Coupons				
wall		5.33E+06		
inside computer		6.51E+06		
corner of floor		13818000		
center of chair		15414000		
keyboard		19173000		
filing cabinet		33600000		
inside computer post-test			ND	
log of geo mean RMC		**7.11**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-12. Test 18 Detailed Results

Office Surface or Object		ClO_2 PPM target = 1000	Contact time =12	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		3.97E+06	1.92E+02	
keyboard		7.80E+06	1.65E+01	ND
chair back	G	7.98E+06	2.00E+01	ND
chair seat		3.55E+06	2.00E+01	ND
pin cushion screen		5.38E+06	1.00E+01	ND
ceiling tile		9.85E+05	1.00E+01	ND
carpet		1.24E+07	1.00E+01	ND
desk top		1.10E+08	1.00E+01	ND
drywall		1.47E+07	1.00E+01	ND
filing cabinet		1.74E+06	9.28E+00	ND
log of geo. mean; log reduction		**6.82**	**1.21**	**5.61**
Total # surface samples (out of 25) = ND				24
avg horiz surf (excl. ceiling tile)		2.33E+07	4.30E+01	
avg vert surf		9.37E+06	1.33E+01	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		2.71E+01	5.88E-03	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		1.23E+07		
inside computer		8.23E+06		
corner of floor		8.40E+06		
center of chair		7.77E+06		
keyboard		1.07E+07		
filing cabinet		1.10E+07		
inside computer post-test			ND	
log of geo mean RMC		**6.98**		

.

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-13. Test 19 Detailed Results

Office Surface or Object		ClO_2 PPM target = 1000	Contact time =12	
	VHP® reset	Pre-decon spore levels	Post-decon spore levels	Post-decon qual. results
catalog		7.87E+05	1.13E+01	
keyboard		2.06E+06	9.97E-01	ND
chair back		1.73E+05	1.50E+00	ND
chair seat		6.34E+05	1.34E+00	ND
pin cushion screen		5.94E+05	1.23E+00	
ceiling tile		1.29E+04	6.00E-01	ND
carpet		1.96E+05	6.15E-01	ND
desk top		4.62E+06	1.10E+01	
drywall		6.98E+05	7.16E-01	ND
filing cabinet		2.32E+06	6.40E-01	ND
log of geo. mean; log reduction		**5.73**	**0.17**	**5.56**
Total # surface samples (out of 25) = ND				22
avg horiz surf (excl. ceiling tile)		1.77E+06	4.32E+00	
avg vert surf		4.88E+05	1.15E+00	
electrical socket swab		G	NG	
keyboard swab			NG	
computer swab			NG	
viacell air sample (CFU/liter)		3.83	2.14E-03	
viacell FB (CFU/sample)		ND		
Ref Measurement Coupons				
wall		6.26E+05		
inside computer		1.08E+06		
corner of floor		9.03E+05		
center of chair		6.26E+05		
keyboard		1.82E+06		
filing cabinet		1.80E+06		
inside computer post-test			ND	
log of geo mean RMC		**6.02**		

Note: all data in CFU/ft^2, except swabs and VHP® reset samples (Growth, No Growth); air samples (CFU/liter)

ND=not detected

Table A-14. Detailed Coupon Efficacy Results

	14B		15		16		17		18		19	
	Pre-decon log CFU value	Post decon result	Pre-decon log CFU value	Post decon result	Pre-decon log CFU value	Post decon result	Pre-decon log CFU value	Post decon result	Pre-decon log CFU value	Post decon result	Pre-decon log CFU value	Post decon result
CT	3.8	ND	3.3	ND	4.1	1	3.3	ND	3.4	ND	3.1	ND
Carpet	4	ND	3.7	ND	4.5	2	3.4	ND	3.2	ND	2.8	ND
WB	3.5	ND	3.2	ND	3.9	1	3.1	ND	2.8	1	2.7	ND
CT	7.3	ND	6.9	ND	7.0	ND	7.0	ND	7.1	ND	6.9	ND
Carpet	7.2	3	7.4	ND	7.2	3	7.2	ND	6.9	ND	7.2	1
WB	6.6	ND	6.7	ND	6.8	3	6.6	ND	6.9	ND	6.0	ND

CT = ceiling tile; WB = painted wallboard paper; ND = no spores detected on any of the 3 coupons tested

If an integer is listed in a "Post decon" column, this reflects number of coupons out of three that had detectable spores.

Appendix B

Detailed Analyses for PC Doctor® Results

PC-Doctor® Service Center™ 7.5 is commercially available software designed to diagnose and detect computer component failures. A complete list of the PC-Doctor® Service Center™ 7.5 diagnostic tests is shown in Table B1.

Table B1. PC- Doctor Tests

Test	Test Description	Tested Subsystem
1	Verify System Information List	SYSTEMS DETECTION
2	RTC Rollover Test	System Board
3	RTC Accuracy Test	System Board
4	Advanced Pattern Test	2048 MB DDR3-SDRAM (1333 MHz)
5	Bit Low Test	2048 MB DDR3-SDRAM (1333 MHz)
6	Bit High Test	2048 MB DDR3-SDRAM (1333 MHz)
7	Nibble Move Test	2048 MB DDR3-SDRAM (1333 MHz)
8	Checkerboard Test	2048 MB DDR3-SDRAM (1333 MHz)
9	Walking One Left Test	2048 MB DDR3-SDRAM (1333 MHz)
10	Walking One Right Test	2048 MB DDR3-SDRAM (1333 MHz)
11	Auxiliary Pattern Test	2048 MB DDR3-SDRAM (1333 MHz)
12	Address Test	2048 MB DDR3-SDRAM (1333 MHz)
13	Modulo20 Test	2048 MB DDR3-SDRAM (1333 MHz)
14	Moving Inversion Test	2048 MB DDR3-SDRAM (1333 MHz)
15	Windows Memory Test	2048 MB DDR3-SDRAM (1333 MHz)
16	Register Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
17	Level 2 Cache Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
18	Math Register Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
19	MMX Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
20	SSE Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
21	SSE2 Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
22	SSE3 Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
23	SSSE3 Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
24	SSE4.1 Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)

25	SSE4.2 Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
26	Stress Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
27	Multicore Test	Intel(R) Core(TM) i5-2500 CPU @ 3.30GHz (CPU:0)
28	Checksum Test	CMOS
29	Pattern Test	CMOS
30	Thermal Cycle Test	Intel(R) HD Graphics Family
31	Shader Rendering DX10 Test	Intel(R) HD Graphics Family
32	Wireframe Shader Rendering Test	Intel(R) HD Graphics Family
33	Shader Rendering Test	Intel(R) HD Graphics Family
34	GPU Pipeline Data Test	Intel(R) HD Graphics Family
35	Transformation and Lighting Stress Test	Intel(R) HD Graphics Family
36	Fixed Transformation and Lighting Test	Intel(R) HD Graphics Family
37	Primary Surface Test	Intel(R) HD Graphics Family
38	Non-Local Video Memory Test	Intel(R) HD Graphics Family
39	Local Video Memory Test	Intel(R) HD Graphics Family
40	SMART Status Test	OS- C: (WDC WD2500AAKX-753CA1)
41	SMART Short Self Test	OS- C: (WDC WD2500AAKX-753CA1)
42	SMART Extended Self Test	OS- C: (WDC WD2500AAKX-753CA1)
43	SMART Conveyance Self Test	OS- C: (WDC WD2500AAKX-753CA1)
44	Linear Seek Test	OS- C: (WDC WD2500AAKX-753CA1)
45	Random Seek Test	OS- C: (WDC WD2500AAKX-753CA1)
46	Funnel Seek Test	OS- C: (WDC WD2500AAKX-753CA1)
47	Surface Scan Test	OS- C: (WDC WD2500AAKX-753CA1)
48	CD Linear Seek Test	HL-DT-ST DVD+-RW GH70N
49	CD Random Seek Test	HL-DT-ST DVD+-RW GH70N
50	CD Funnel Seek Test	HL-DT-ST DVD+-RW GH70N
51	CD Linear Read Compare Test	HL-DT-ST DVD+-RW GH70N
52	CD Audio Test	HL-DT-ST DVD+-RW GH70N
53	CD-R Read Write Test	HL-DT-ST DVD+-RW GH70N
54	CD-RW Read Write Test	HL-DT-ST DVD+-RW GH70N
55	DVD+R Read Write Test	HL-DT-ST DVD+-RW GH70N
56	DVD-RW Read Write Test	HL-DT-ST DVD+-RW GH70N
57	DVD Linear Seek Test	HL-DT-ST DVD+-RW GH70N
58	DVD Random Seek Test	HL-DT-ST DVD+-RW GH70N
59	DVD Funnel Seek Test	HL-DT-ST DVD+-RW GH70N
60	DVD Linear Read Compare Test	HL-DT-ST DVD+-RW GH70N
61	AVI Interactive Test	AVI Test

62	Monitor Interactive Test	DELL E1911 (Generic PnP Monitor)
63	Keyboard Interactive Test	HID Keyboard Device
64	Mouse Interactive Test	HID-Compliant Mouse
65	Network Link Test	Intel(R) 82579LM Gigabit Network Connection
66	TCP/IP Internal Loopback Test	Intel(R) 82579LM Gigabit Network Connection
67	Network External Loopback Test	Intel(R) 82579LM Gigabit Network Connection
68	Configuration Test	PCI Bus
69	PCI Express Status Test	High Definition Audio Controller
70	PCI Express Status Test	Intel(R) 6 Series/C200 Series Chipset Family PCI Express Root Port 1 - 1C10
71	PCI Express Status Test	Intel(R) 6 Series/C200 Series Chipset Family PCI Express Root Port 3 - 1C14
72	Standby Test	Standby/Hibernate
73	Hibernation Test	Standby/Hibernate
74	Internal Register Test	Intel(R) Active Management Technology - SOL (COM3)
75	Internal Control Signals Test	Intel(R) Active Management Technology - SOL (COM3)
76	Internal Send and Receive Test	Intel(R) Active Management Technology - SOL (COM3)
77	Internal Register Test	Communications Port (COM1)
78	Internal Control Signals Test	Communications Port (COM1)
79	Internal Send and Receive Test	Communications Port (COM1)
80	USB Port Test	USB Port Test
81	Rough Audio Test	Realtek High Definition Audio
82	Sound Interactive Test	Realtek High Definition Audio
83	USB Status Test	USB Hub 1
84	USB Status Test	USB Hub 2
85	USB Status Test	USB Test Key
86	USB Status Test	Dell USB Entry Keyboard
87	USB Status Test	USB Optical Mouse
88	USB Status Test	USB Storage Device
89	USB Status Test	USB Storage Device
90	USB Status Test	USB Storage Device
91	USB Status Test	USB Storage Device
92	USB Status Test	USB Storage Device
93	USB Status Test	USB Storage Device
94	USB Status Test	USB Storage Device

The PC-Doctor® Service Center™ 7.5 protocol was developed to have an industry-accepted standard method of determining pass versus failure of the computer subsystems. PC-Doctor® Service Center™ 7.5 functionality testing was conducted on each computer pre-fumigation, one day post-fumigation, then monthly for the next year with exceptions due to budget constraints. This testing provided valuable information about the extent and time dependence of the degradation of these computers following the various fumigation scenarios. All computers were kept under ambient laboratory conditions, in which humidity was not strictly controlled.

When conducting a functionality test on a computer, if any particular sub-system check (out of the 94 performed) failed the first time, the computer was tested a second time to correct for possible human error. A test that failed the second time was labeled "Fail". If the test failed the first time but passed the second time, it was labeled "Pass2". There were certain instances when the computer did not allow certain tests to be run. These were listed as "False-Fail", because though the test was not run, it was considered a failure since the test should have been able to run. For tabulation, a score of 1,000 was assigned to each "Fail" and "False-Fail", while a "Pass2" received a score of 1. Some test dates have a total of only 93 tests; in this case, the results were not manually recorded due to operator error. There was no score assigned to known operator error. During each pre- and post-fumigation testing period, a total PC-Doctor® score was the sum of the scores from each of the 94 tests. A score of 0 indicated a computer that passed all tests, while a score of 94000 would indicate a computer that failed all tests.

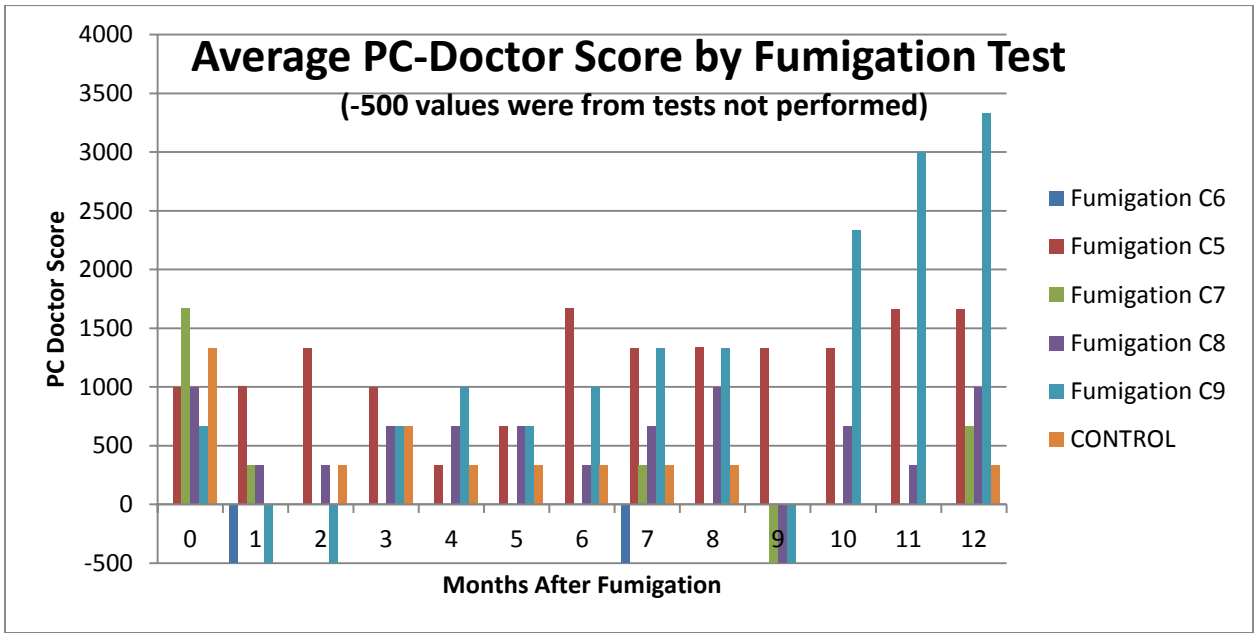

Figure B1. Average PC-Doctor Score over time*.

*Computer B (JW08) value for Month 0 Fumigation C5 removed in this graph as an outlier

Figure B1 shows the average score of three replicate computers for each month. Some computers were not tested each month, and they were assigned a value of -500 in Figure 1 to distinguish a 0 (All pass) from a null value (not performed). Table B2 provides a total of all incidents of PC-Doctor® Service Center™ 7.5 tests that received a "Fail." For each test condition, the results are shown for each of the computers that underwent year-long testing.

Table B2. Total "Fail" Results For Year-Long Test Period

Fumigation Technology	None	200 ppmv ClO_2, 6 hr. (C6)	100 ppmv ClO_2, 12 hr. (C5)	200 ppmv ClO_2, 8 hr. (C7)	300 ppmv ClO_2, 4 hr. (C8)	300 ppmv ClO_2, 8 hr. (C9)
Computer A	7	1	15	4	2	30
Computer B	6	0	36+42[a]	3	3	0
Computer C	0	0	7	5	22[b]	27[b]

a = This computer did not record the results of some tests for one test day
b = This computer never detected two USB ports, which therefore always failed. This was a preexisting condition.

Some of these failures were present before the fumigation, and so can be ruled out as an effect of fumigation. Table B3 shows the results from Table B2, with pre-existing results removed.

Table B3. Total "Fail" Results with Pre-Existing Failures Removed

Fumigation Technology	None	200 ppmv ClO_2, 6 hr. (C6)	100 ppmv ClO_2, 12 hr. (C5)	200 ppmv ClO_2, 8 hr. (C7)	300 ppmv ClO_2, 4 hr. (C8)	300 ppmv ClO_2, 8 hr. (C9)
Computer A	7	0	15	4	2	30
Computer B	6	0	36+42[a]	1	3	0
Computer C	0	0	7	3	1	0

a = This computer, JW08, did not record the results of 42 tests for one test day

Fumigation at 200 ppmv ClO_2 for 6 or 8 hours (C6 and C7) and 300 ppmv ClO_2 for 4 hours (C8) had no measureable effect on the function of the computers. The effects of the 12 hour 100 ppmv ClO_2 (C5) and the 8 hour 300 ppmv ClO_2 (C9) fumigation are more difficult to interpret. In both cases, at least one computer performed as well as or better than the control computers, and at least one computer performed worse. With such a small sample size, it is impossible to say with any statistical certainty that poorer performance was a result of the exposure to fumigation conditions.

Table B4 lists of all failures as a function of subsystem. The failures are dominated by USB failures, which, as stated before, do not seem to be a result of fumigation, given that they were present before fumigation.

Table B4. Vulnerable Subsystems

Test Number	Test Description	Total Number of Events		
		Pass2	Fail	False Fail
41	SMART Short Self Test		2	
42	SMART Extended Self Test		3	
43	SMART Conveyance Self Test		3	
47	Surface Scan Test		3	
48	CD Linear Seek Test		1	
52	CD Audio Test	4	7	
53	CD-R Read Write Test	4	0	
54	CD-RW Read Write Test	1	0	
55	DVD+R Read Write Test	9	15	7
56	DVD-RW Read Write Test	4	2	
63	Keyboard Interactive Test	1	0	
65	Network Link Test	0	1	
67	Network External Loopback Test	2	1	
72	Standby Test	16	2	
73	Hibernation Test	9	3	
80	USB Port Test	5	18	
81	Rough Audio Test	9	2	
82	Sound Interactive Test	1	0	
92	USB Status Test	1	1	
94	USB Status Test	1	0	1
95	USB Status Test	1	0	28
96	USB Status Test	1	7	61

*Computer B (JW08) value for Month 0 Fumigation C5 removed in this table as an outlier

EPA
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Environmental Protection
Agency

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