Analysis of Chlorine Dioxide Remediation of Washington, DC *Bacillus anthracis* Contamination

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EXECUTIVE SUMMARY

This report provides a scientific analysis of chlorine dioxide gas testing and remediation efforts conducted or sponsored by the U.S. Environmental Protection Agency (USEPA) in the Washington, DC, area in response to the anthrax attacks in October 2001. Commissioned by the USEPA Region III during the response phase to these attacks, this report summarizes and evaluates the available data from tests conducted by USEPA at a trailer test facility at the USPS Brentwood Processing and Distribution Center (hereafter referred to as the Brentwood P&DC), from the fumigation of the Daschle suite and part of the heating, ventilation, and air conditioning (HVAC) system of the Hart Senate Office Building (HSOB), from additional USEPA trailer tests in Beltsville, Maryland, and from USEPA-sponsored tests at the U.S. Army Dugway Proving Ground, Utah. Finally, this report offers key findings derived from all of these data concerning the effectiveness of chlorine dioxide gas for inactivating *Bacillus anthracis* spores under laboratory and field conditions.

The introduction of *B. anthracis* spores into the U.S. Mail distribution system in the fall of 2001 presented a host of challenges to many government and civilian institutions. Envelopes containing approximately one to two grams of dry, weaponized *B. anthracis* spores resulted in contamination of multiple buildings in the Capitol Hill area. Levels of contamination ranged from just detectable levels to visible powder on the floor in Senator Daschle's suite. The facts that 1 gram contains as many as 10^{12} spores and an infective inhaled dose may range from less than 10 spores to tens of thousands of spores presented some unique challenges for the cleanup operations.

The USEPA assumed overall responsibility for the remediation of buildings and artifacts within the Capitol Hill region. Localized small-scale remediation efforts were performed by high efficiency particulate air (HEPA) vacuuming, decontamination foam, and chlorine dioxide dissolved in water. Large-scale remediation was conducted by fumigating with chlorine dioxide gas in specific locations in the HSOB. This effort represented the first time chlorine dioxide gas was used for the destruction of *B. anthracis* spores outside of a laboratory and for decontamination on this scale.

Chlorine dioxide gas was selected for fumigation of the Daschle suite and a section of the HVAC system in the HSOB after careful consideration of several gaseous or vaporized alternative chemicals, including paraformaldehyde (heated into formaldehyde gas), ozone, ethylene oxide, and hydrogen peroxide vapor. While these alternative chemical decontaminants were all known to have potential effectiveness against *B. anthracis* spores, an interagency committee of advisors selected chlorine dioxide gas based on an objective evaluation using specific criteria that are described in paragraph 2.1.4.

Prior to the remediation of the HSOB, USEPA conducted a series of fumigations with chlorine dioxide gas in a trailer located at the Brentwood PD&C, which has been subsequently renamed the "Joseph Curseen-Thomas Morris P&DC." The purpose of this testing was to determine the most effective combination of gas concentration, temperature, relative humidity, and contact time for fumigation of the HSOB Daschle suite and HVAC system. After the building remediation efforts were completed, USEPA conducted additional tests in a trailer at Beltsville, Maryland, and funded another study at the U.S. Army Dugway Proving Ground, Utah, on the efficacy of chlorine dioxide gas on live *B. anthracis* and several surrogate *Bacillus* species.

This report provides a scientific analysis of the results of all of the tests that were conducted or sponsored by USEPA to determine the most effective conditions for using chlorine dioxide gas to inactivate *B. anthracis* spores.

Finally, the authors wish to note that the historic, successful remediation of the HSOB is a tribute to the professionalism, dedication and hard efforts of all of the members of the remediation team led by the Capitol Police Board (CPB), the USEPA, Department of Defense, other federal agencies, and the Incident Commander, along with many local government fire and rescue teams.

Key Findings

1. Chlorine dioxide is an effective agent for the destruction of bacterial spores both as a gas and when dissolved in water. The data generated by the USEPA team from the use of chlorine dioxide gas at the HSOB and in separate tests provide strong evidence for the sporicidal effects of the oxidizer. The results obtained are supported by literature values determined in laboratory settings.

2. Initial testing with chlorine dioxide gas in a remediation test trailer at the Brentwood P&DC was crucial for the identification of successful operational parameters. These tests set the minimum levels for temperature, relative humidity, gas concentration, time of exposure, and indicator organisms to achieve the desired level of killing efficiency (see Key Finding 9 for details).

3. Insufficient time was allowed to train the personnel handling the spore strips. This training includes both the physical handling (placement, labeling, collection) as well as the subsequent laboratory culture analysis.

4. There was no confirmation of the type of organism cultured in positive spore strip samples taken during HSOB remediation. Therefore, one cannot conclude that the growth from indicator spore strips was from the indicator organism or a contaminant. Later analyses of cultured organisms from positive cultures from the Beltsville test trailer demonstrated that the organism contained in the spore strip culture frequently was not the original organism cultured, indicating a secondary source of contamination.

5. Inconsistent Steri-chart results confounded analysis within the offices of the HSOB. Approximately one-third of the Steri-chart series demonstrated positive growth at one level, negative growth at the next higher level followed by positive growth at higher levels. These results were consistent with irregularities on the spore strip formulations.

6. Ineffective communications with the analytical laboratory resulted in the first 2 days of culture testing for spore strips containing the test organism *Bacillus stearothermophilis* being conducted at 37°C rather than at 60°C, the optimal temperature for this organism. This error, combined with the fact that positive cultures were not characterized, puts the additional information provided by this organism in question.

7. There was no consistent correlation between efficiency of kill and spatial placement of spore strips, for example, vertical surfaces were not remediated better or worse than horizontal surfaces. This was true for the HSOB as well as the trailer chambers.

8. Variability in the environmental conditions in the HVAC system, primarily the wide variation in relative humidity, complicated the analysis of the results, but the overall efficiency of both fumigations within the HVAC system was high.

9. Based on review of all of the data presented in this report, the minimum target gas concentration (C=750 parts per million [ppm]) and total contact time (CT) (T=12 hours) for a total CT of 9,000 ppmv-hrs appear to be just as important as the minimum temperature (greater then 75°F) and relative humidity (75%) to assure that chlorine dioxide kills bacterial spores.

10. Independent experimentation conducted at the West Desert Test Center at U.S. Army Dugway Proving Ground, which was funded by USEPA, indicates that relative humidity is a critical factor in chlorine dioxide remediation of *B. anthracis* spores. In addition, these tests were conducted on live *B. anthracis* as well as other surrogate spores on glass and paper. The results, presented in *Appendix 1,* provide further evidence that the conditions utilized for fumigation operations at the HSOB were effective against *B. anthracis* spores.

11. Use of spore strips and Steri-charts to measure the effectiveness of the use of chlorine gas to fumigate the Daschle suite and to fumigate a portion of the HVAC system indicated that those fumigations were not completely successful in reaching the target level of kill efficiency (10^6 reduction in spores) in all spore strips. However, the use of liquid chlorine dioxide to treat locations where spore strips were positive, and subsequent clearance sampling conducted in those locations and throughout the entire HSOB demonstrated **no growth** on all environmental samples. On that basis, the overall remediation was declared to be successful and the HSOB was cleared for re-opening.

LIST OF ACRONYMS

AHU	air handling unit
AOC	Architect of the Capitol
ATSDR	Agency for Toxic Substances and Disease Registry
Bs	*Bacillus subtilis*
BSL-3	Biosafety level 3
Bst	*Bacillus stearothermophilus*
CBIRF	U.S. Marine Corps Chemical and Biological Incident Response Force
CDC	Centers for Disease Control
Cfus	Colony Forming Unit
CHPPM	U.S. Army Center for Health Promotion and Preventive Medicine
CID	Criminal Investigation Division
COC	Chain of Custody
CPB	Capitol Police Board
CT	concentration over time
DFU	dry filter unit
DSOB	Dirksen Senate Office Building
ERRS	Emergency and Rapid Response Services
ERT	Emergency Response Team
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
HCl	hydrochloric acid
HEPA	high efficiency particulate air
HSOB	Hart Senate Office Building
HVAC	heating, ventilation, and air conditioning
IAP	The Incident Action Plan
IATA	International Air Transport Association
ICC	Incident Command Center
ICS	Incident Command System
$NaClO_2$	sodium chlorite (-)
NaOCl	sodium hypochlorite
NIIMS	National Interagency Incident Management System
NIOSH	National Institute for Occupational Safety and Health
NMRC	Naval Medical Research Center
OSC	On-Scene Coordinator

P&DC	Process and Distribution Center
ppb	parts per billion
ppm	parts per million
ppmv	parts per million volume
PPE	personal protective equipment
RH	relative humidity
SOP	standard operating procedure
START	Superfund Technical Assessment and Response Team
SwRI®	Southwest Research Institute®
TAGA	trace air gas analyzer
TSB	trypticase soy broth
UC	University of California
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
USCG	U.S. Coast Guard
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency
USPS	U.S. Postal Service

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1. INTRODUCTION AND SCOPE OF ANALYSIS

The purpose of this report is to conduct a scientific analysis of chlorine dioxide remediation efforts conducted by the U.S. Environmental Protection Agency (USEPA) in response to anthrax attacks in the Washington, DC, area in 2001. The report was commissioned by the USEPA Region III On-Scene Coordinator (OSC) for Washington operations, Mr. Richard Rupert, during the response phase to the anthrax attacks. This report represents a historical analysis of available data from tests conducted by USEPA at a trailer test facility at the U.S. Postal Service (USPS) Brentwood Processing and Distribution Center (P&DC), the fumigation of the Hart Senate Office Building (HSOB), additional USEPA trailer tests in Beltsville, Maryland, and from USEPA-sponsored tests at the U.S. Army Dugway Proving Ground, Utah.

Interviews were conducted with key USEPA individuals as well as support contractors and laboratory analytical teams. This report provides an analysis of the effectiveness of chlorine dioxide gas for killing *Bacillus anthracis* spores and fumigation of the HSOB. The opinions expressed are those of the authors and not of USEPA; however, this document has been reviewed by USEPA staff for completeness and accuracy. All individuals involved in the post attack remediation performed in an exemplary, professional manner. The situation was new to all involved and new issues arose at every turn of events. The success of the overall operations is demonstrated by the fact that no one in the Capitol Hill complex developed symptoms of anthrax and that all buildings were treated, cleared, and returned to full operations in only 3 months.

1.1 Initial Response to Capitol Hill Anthrax Contamination

On Monday, October 15, 2001, an envelope addressed to Senator Daschle and containing a fine powder of *B. anthracis* (anthrax) spores was delivered to the HSOB and opened by a Senate employee. The Capitol Hill police were notified and the Capitol Police Bomb Squad responded to the scene in Level C personal protective equipment (PPE). Members of the squad sampled the contents of the envelope using an antibody-based handheld assay and obtained a positive result within minutes. Agents from the Federal Bureau of Investigation (FBI) responded to initiate a criminal investigation and confiscated the envelope and its contents as evidence.

On October 16, the USEPA was notified and an OSC from Region III responded. On October 17, the HSOB was closed and additional OSCs and assessment teams were mobilized from Region III to the Capitol Hill area. Members of the USEPA's Criminal Investigation Division (CID) were sent to assist the FBI with collection of additional evidence and investigation. In addition, USEPA Superfund Technical Assessment and Response Team (START) and Emergency and Rapid Response Services (ERRS) contractors were mobilized to perform sampling and contain contaminated areas, to conduct and monitor cleanup activities, and to provide technical support to the USEPA. The U.S. Coast Guard (USCG) also responded at the request of USEPA. The USCG, Federal Emergency Management Agency (FEMA) and the USEPA recommended implementing the National Interagency Incident Management System (NIIMS) Incident Command System (ICS) to manage the response. A formal structure was set in place on October 20, 2001, and an Incident Command Center (ICC) was established.

1.2 Response Challenges

Anthrax contamination of Capitol Hill was an unprecedented event in terms of scope, complexity and nature of the threat that presented responders with extraordinary challenges. The incident response involved coordination of more than 50 organizations. This was the first time that the USEPA, like many other organizations involved, had to address remediation of buildings contaminated with a biological agent, in particular, an extremely hardy agent such as anthrax spores. Thus, technologies and procedures for sampling and remediation had to be identified and devised with little prior experience to draw from. The potentially contaminated area was large and widespread, dramatically complicating the cleanup effort. Finally, the speed of response was of utmost importance in order to minimize the impact on the displaced population from the legislative branch of the U.S. Government, a group of individuals commanding high levels of authority.

2. SAMPLING PLANS AND PROCEDURES

Sampling was conducted in three phases: **screening sampling** to identify the extent of contamination, **characterization sampling** to identify areas for remediation and appropriate methods, and **clearance sampling** to confirm that remediation efforts were adequate. Surface and air samples were collected during all three sampling phases. Three types of surface samples were taken using swabs, wipes, and high efficiency particulate air (HEPA) vacuums. Swab and wipe samples were used to take samples from nonporous surfaces. Swabs were used to sample distinct locations. Wipes were used for composite samples taken from larger surface areas. HEPA vacuums were used to sample porous surfaces and to verify remediation effectiveness.

On October 17, personnel from the National Institute for Occupational Safety and Health (NIOSH) performed initial sampling with assistance from the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) and the U.S. Marine Corps Chemical and Biological Incident Response Force (CBIRF). USEPA teams collected screening samples for analysis. Personnel from the Architect of the Capitol (AOC) provided logistical and reconnaissance support to the USEPA. Building reconnaissance (taking photographs and validating building architectural plans) was conducted between October 16, 2001 and January 2, 2002. Objectives of this exercise were to become familiar with the layout of the building, to observe the construction and accessibility of the plenum to sampling points within Senate suites, to identify mail-handling locations, and to videotape areas to be used to prepare sampling teams.

Sampling equipment was initially provided by NIOSH and later by CHPPM. In addition, CHPPM was responsible for receiving samples from sampling teams, labeling them, and ensuring chain of custody. Next, the samples were given to the Capitol Police who shipped them to one of two analytical laboratories: the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) or the Naval Medical Research Center (NMRC). While both USAMRIID and NMRC provided excellent technical support to the overall operations, there was not an established extraction and analysis method employed by both laboratories. This led to two areas of complication: (1) there was no way to get a quantifiable level of contamination due to the complications of extraction and analysis and (2) the two laboratories used different reporting criteria. The NMRC reported a number for colony formation, while USAMRIID did

not. For future operations, a standardization of both sample collection and analysis is recommended.

Sampling was performed along congressional mail routes identified by FBI investigators to determine other potential areas of contamination. The mail route was traced back from the HSOB to the Dirksen Senate Office Building (DSOB) where the mail for the HSOB is processed. Prior to arrival at the DSOB, Senate mail is held in the P Street Warehouse, a restricted mail inspection facility overseen by the Capitol Police. It is sent to the P Street Warehouse from the Brentwood Post Office, a large USPS mail processing and distribution center for the Washington, DC, area. The air handling unit (AHU) and heating, ventilation and air conditioning (HVAC) systems were sampled in the vertical zone of the HSOB where the contaminated letter was delivered. In addition, the mail drops in the Dirksen Building were sampled.

At the time of the screening sampling, there were no formal sampling procedures in place. Centers for Disease Control (CDC) guidance was followed and sampling plans were written on construction plans of the buildings provided by the AOC. These plans were provided to sampling teams. In mid to late October, USEPA assumed the task of verification sampling from NIOSH. NIOSH continued to prepare plans for characterization sampling.

2.1 Sampling Plans

2.1.1 Difficulties with Sampling Plans and Procedures. No standard operating procedures (SOPs) were available for characterization and clearance sampling. Likewise, a conceptual model to predict distribution of spores and the extent of contamination had to be generated "from scratch" under extreme time pressure. In addition, the urgency of the response required that personnel work long hours under constant strain. Inaccuracies in the floor plan complicated planning and implementation of sampling and remediation efforts. Precautions taken to minimize the potential for cross-contamination slowed the pace of sampling, as did requests for items from specific offices. Finally, communication between planners and sampling personnel was poor. Rotating day and night shift and fluctuations in personnel being mobilized and demobilized made it difficult in some cases for samplers to determine where previous samples were taken and has hindered compilation of a historical perspective of the sampling effort.

In spite of these issues, the sampling and remediation activities were successful overall based on the fact that all clearance samples showed no growth in any areas previously contaminated with *B. anthracis*. In addition, no one has presented with symptoms of anthrax since buildings on Capitol Hill were remediated and cleared for reoccupancy. This is clearly the best measure of the success for the response and remediation activities. The high level of cooperation between multiple agencies, their contractors, and scientific advisors was also a success. Over the course of the response, sampling objectives were clarified and plans became more consistent, thereby improving implementation and interpretation of results. Over 9,000 samples were collected throughout the course of the response. The process involved managing large quantities of data and tracking samples through collection, analysis, and interpretation that involved numerous U.S. Government agencies, contractors, and locations. In the end, only a few samples were unaccounted for.

2.1.2 Data Management. The Capitol Police Board (CPB) contracted in late October to develop and employ a method to manage the sampling data. Initially, sampling results were scored positive or negative and stored in a Microsoft® Excel spreadsheet. The results were then overlaid on building plans obtained from the AOC and scanned into the system. This display was somewhat awkward, especially when multiple samples were taken from a particular location. In mid-November, an improved data system was deployed that employed barcodes for samples and logged their positions using a Geographic Information System. This meant that the chain of custody to be logged automatically, eliminating possible errors due to manual data entry. In addition, using the new system, the data could be shared using a secure Web site. This allowed the analysis laboratories to update sample information remotely, thereby reducing the time between sampling and reporting of results. Initially, it took four to five days to receive results. By early December, initial verbal results could be received within a day.

Data management was a key operational parameter for the sampling operations, as well as for the validation of remediation operations. Multiple thousands of samples were collected both pre and post remediation. The thousands of spore strips used for remediation validation presented an additional burden on the data management system. The team functioned well in a new data management environment in a dynamic time. The data management of future operations would benefit from a standardization of these types of operations.

2.1.3 Remediation Technology Options. USEPA personnel drafted a list of options for remediation of the HSOB on October 19, 2001, and enlisted a team of advisors to compare remediation methods in terms of effectiveness versus possible damage to exposed surfaces. The extent of total contamination was unknown, and the pressure to conduct remediation operations was real. Alternatives considered included ozone, ethylene oxide, paraformaldehyde, ClO_2 gas, ClO_2 dissolved in water, bleach, and reactive foams such as EasyDECON™ foam and L-Gel, and hydroxyl radicals. The committee determined that ClO_2 gas was most likely to destroy the spores present in high concentration in the contaminated suite and HVAC system in the HSOB with minimal damage and disruption.

The remediation alternatives considered for application in the affected buildings on Capital Hill following *B. anthracis* contamination. Information on the application of these methods in an office building setting was limited. Effectiveness and safety were the ultimate drivers. Key considerations were:

- Minimal disruption of the target areas
- High efficiency of spore kill (six orders of magnitude or greater)
- No residual toxicity
- Ease of use
- Documented killing efficiency
- Minimal secondary chemical reactions that decrease killing efficiency.

Numerous remediation technology alternatives were available to the USEPA and each technical approach had its own set of advocates. It was necessary for the USEPA to define these key criteria that were important for a critical evaluation of the available technical approaches. Table 1 summarizes the criteria and findings that allowed comparisons of the different technical approaches and their unique attributes for the task at hand.

Table 1. Criteria for Evaluating Remediation Options in Response to Anthrax Contamination on Capitol Hill

Categories of Remediation Agents	Remediation Option	Requirements	Disruption of Target Area	Efficiency of Spore Kill	Residual Toxicity	Ease of Use	Ability to Document Killing Efficiency	Reactions that Decrease Killing Efficiency
Liquid	Bleach (sodium hypochlorite)	Solid surface for application	Most papers and electronic components are destroyed. Porous surfaces such as upholstery and carpet are negatively affected	10^6 kill documented for bacteria spores	Free chlorine	Manual or spraying. Sufficient contact time on vertical surfaces is an issue	Difficult to tell whether material is destroyed or simply removed by washing	Secondary chemical reactions should not be a problem if there is sufficient concentration and contact time
	Chlorine Dioxide	Solid surface for application	Most papers and electronic components are destroyed. Porous surfaces such as upholstery and carpet are negatively affected	10^6 kill documented for bacteria spores	Gaseous chlorine dioxide	Manual or spraying. Sufficient contact time on vertical surfaces is an issue	Difficult to tell whether material is destroyed or simply removed by washing	Secondary chemical reactions should not be a problem if there is sufficient concentration and contact time
Reactive Foams	EasyDECON™ Foam	Solid or porous surfaces for application	Protocol is to discard all loose material. Potential for damage to electronics is high	10^6 kill claimed on anthrax spores with a contact time of 1 hour	No residual toxicity but there is a substantial residue that must be removed	USEPA teams who remediated the Ford Mail Room gave negative ratings	Difficult to tell whether material is destroyed or simply removed by washing	No documented interference of killing activity
Fumigation Options	Chlorine Dioxide	Suitable for nonporous and porous surfaces	Minimal disruption of target area. Oxidative reactions may result in discoloration of some materials	10^6 kill documented for bacterial spores	No residual toxicity[a,b]	Gas must be generated onsite. Area must be isolated	Spore strips can be used to validate killing efficiency	None documented

Table 1. Criteria for Evaluating Remediation Options in Response to Anthrax Contamination on Capitol Hill (continued)

Categories of Remediation Agents	Remediation Option	Requirements	Disruption of Target Area	Efficiency of Spore Kill	Residual Toxicity	Ease of Use	Ability to Document Killing Efficiency	Reactions that Decrease Killing Efficiency
Fumigation Options (continued)	Paraformaldehyde (heated to create formaldehyde gas)	Suitable for nonporous and porous surfaces	Minimal disruption of target area. Residual chemicals on surfaces make subsequent cleanup required.	10^6 kill documented for bacterial spores, including *B. anthracis*	Potentially toxic residue remaining on all surfaces[a,b]	Gas must be generated onsite. Area must be isolated	Spore strips can be used to validate killing efficiency	None documented
	Ozone	Suitable for nonporous and porous surfaces; however, is relatively unstable and may not be suitable for large volumes.	Minimal disruption of target area	10^6 kill documented for bacterial spores, including *B. anthracis*	No residual toxicity	Gas must be generated onsite. Area must be isolated	Spore strips can be used to validate killing efficiency	None documented
	Vapor Hydrogen Peroxide	Suitable for nonporous and porous surfaces	Minimal disruption of target area	10^6 kill documented for bacterial spores, including *B. anthracis*	No residual toxicity	Gas must be generated onsite. Area must be isolated	Spore strips can be used to validate killing efficiency	Agent inactivated by cellulose-containing compounds, e.g., paper products

Notes:

[a] Personnel exposure limits: chlorine dioxide 0.1ppp; formaldehyde 0.75ppm; vapor hydrogen peroxide 1.0ppm
[b] Immediate danger to life and health: chlorine dioxide 5.0ppm; formaldehyde 20ppm; vapor hydrogen peroxide 75ppm

The large volume of building space requiring treatment as well as the widespread contamination dictated that fumigation be the primary mode of remediation. Table 1 highlights gases and vapors documented to be highly effective for inactivating spores. Of the available fumigants tested against spores, chlorine dioxide appeared to be the most promising. Formaldehyde was known to be genotoxic and cause cancer in animals. In addition, it would leave a residue and would require off gassing for an extended period of time. Vapor hydrogen peroxide was known to be an effective fumigant but was reported to inactivate on contact with porous surfaces such as cement and paper. USEPA's initial desires were to leave as much of the office material intact as possible. Ozone, while effective, was known to be relatively unstable and was not considered a viable candidate for large areas. Many considerations were given to the numerous alternatives including discussions with industry and scientific experts. The consensus was that chlorine dioxide was the best choice based on the currently available information.

2.1.4 Decision to Use ClO_2 Gas. On October 25, 2001, USEPA personnel announced their decision to use ClO_2 gas and liquid to conduct remediation operations within the HSOB. The CPB agreed with the decision and the USEPA OSC drafted a proposal to use ClO_2 gas as the fumigant of choice. A peer review panel was enlisted on October 31, 2001, and given 48 hours to respond. They were asked to answer the following questions: (1) Is fumigation appropriate for remediation of the HSOB? (2) Is treatment with ClO_2 gas appropriate? and (3) Should the entire building be fumigated? USEPA compiled and reviewed the comments and announced on November 4, 2001, that the reviewers agreed that ClO_2 gas was the best fumigation option for remediation of the HSOB. Many, however, expressed uncertainty about fumigating the entire building. Thus, USEPA chose to scale back the operation and initiate a step-wise approach that would first use ClO_2 gas fumigation to remediate the contaminated suite and then treat other areas as needed. This decision created a need to perform detailed characterization sampling in many rooms and common areas of the HSOB that considerably extended the timeline for remediation.

2.2 Remediation Phase

On October 24, 2001, the USCG established a demobilization unit to initiate a plan to transition from an emergency response (incident organization response) to a remedial phase. The transition was completed on November 13, 2001, when all contaminated areas had been identified and isolated. The Incident Action Plan (IAP) used in the emergency phase was replaced by the use of building-specific plans in the remediation phase. Once the transition was made to the Remedial Phase Organization, the Incident Commander managed remediation of four buildings. OSC Rupert was the USEPA Project Coordinator for this phase. In this capacity he facilitated USEPA issues related to policy and programming, in particular, scope of work to be performed by USEPA. OSCs coordinated activities with their support elements (for example, Capitol Hill Police and AOC) and Congressional representatives through the USEPA Project Coordinator. In addition, the USEPA Project Coordinator approved all activities, plans, and project time lines.

The sampling approach defined three primary contamination pathways: mail delivery route, personnel traffic, and AHU (return and supply sides) and HVAC systems. Sampling plans also included HSOB common areas such as hallways, atriums, and restrooms. Calculations indicated that a huge number of spores could have been released upon opening of the anthrax-laden letter. Thus, every room in every suite in the area to which the letter was delivered was sampled extensively in the characterization phase.

Planners consulted with asbestos experts to predict spore transport and settling because the size (1 to 10 micron) and transport properties of the spores were thought to be similar to asbestos particles. In fact, the FBI determined that the spore preparation had been formulated using silicates (that is, weaponized). The silicate preparation was thought to allow the spores to remain airborne longer and to make the spores easier to reaerosolize. This information prompted a series of tests, conducted between November 10, 2001, and November 24, 2001, to determine whether the spores were likely to reaerosolize. The test results indicated that the spores, particularly those in the respirable range (less than 5 micron diameter) were likely to reaerosolize. The results raised concern about possible migration of the spores and related concerns about decontamination methods and appropriate PPE levels. In addition, all HSOB HVAC systems were sampled along with secondary contamination routes (those resulting from indirect contact with contaminated mail).

After implementation of the Remedial Phase Organization in mid-November, OSCs were assigned to oversee building-specific plans and activities. Sampling activities were conducted simultaneously at multiple buildings and by November 20, 2001, it was determined that all buildings except the HSOB were adequately characterized.

Clearance sampling plans were prepared to assess the overall effectiveness of remediation efforts. As with other phases of the response, standard procedures and protocols were not available. Thus, the USEPA, in consultation with a biostatistician, developed a method to collect numerous surface samples in each room that was remediated. The plan required that 10 to 20 percent of the horizontal surfaces in a particular area be sampled following remediation. A sampling grid was used on the floors, walls, and in the HVAC system. Vertical, nonporous surfaces rarely exhibited contamination (only two wall samples tested positive during the entire course of the response.) Thus, only one composite screen sample and one wall sample were collected from vertical surfaces during the verification phase. Ultimately, over 400 clearance samples were collected in the suite to which the contaminated envelope was delivered.

The Agency for Toxic Substances and Disease Registry (ATSDR) and NIOSH developed an air sampling plan from established asbestos protocols. A variety of techniques were used, to include mixed cellulose ester membrane (gelatin) filters to collect air samples for 45 minutes, dry filter units (DFUs), and a cascade impactor sampler (Anderson Sampler). Air samples collected using DFUs generally processed 2.5 room volumes of air at a rate of 900 liters per minute. The cascade impactor was used to obtain samples to be cultured. Samples were also collected on agar plates exposed to the air for 45 minutes or more.

2.2.1 Outstanding Issues Relative to Chlorine Dioxide Fumigation. There exists a wealth of data demonstrating the ability of chorine dioxide to sterilize water, bleach paper pulp, disinfect medical equipment, and inactivate multiple types of gram positive bacterial spores in controlled laboratory conditions. However, there was no data on the ability of chlorine dioxide to inactivate dried *B. anthracis* spores in an office environment. Even though some of the surrogate spore species contained in spore strips used for measuring the effectiveness of fumigation were regarded as "tougher" than the *B. anthracis* spores, this remained a concern. Dr. Amy Alving of Defense Advanced Research Projects Agency provided financial support for laboratory analysis of the effectiveness of chlorine dioxide gas for inactivating *B. anthracis* spores. The preliminary results supported laboratory evaluation of other *Bacillus* spores using the gas. The gas was effective at temperatures above 75°F and relative humidity above

75 percent with a gas concentration in excess of 4,000 ppm for at least a 1-hour duration. Subsequent detailed analysis conducted at Dugway Proving Ground, funded by the USEPA in Colorado, supported these findings (see *Appendix 1*).

Although laboratory results supported the effectiveness of chlorine dioxide against *Bacillus* spores, large scale testing data was lacking. In order to fill this critical void, the USEPA Emergency Response Team (ERT) established a trailer testing facility for the use of chlorine dioxide in a non-laboratory setting.

2.2.2 Establishment of Brentwood P&DC Test Facility. USEPA selected Sabre Oxidation Technologies, a company with expertise in the use of ClO_2, as the contractor to supply ClO_2 for fumigation remediation options. During the time when various remediation alternatives were being considered, the USEPA ERT coordinated between Sabre Oxidation Technologies, the Capitol Police, and the USPS to construct a testing facility at its Brentwood P&DC to determine conditions necessary for the remediation of *B. anthracis* spores. This was a key event in the overall remediation strategy because gaseous ClO_2 had never been used as a decontaminant in an office building setting. A large body of information was available documenting the sporicidal capabilities of chlorine dioxide under strict laboratory conditions; however, testing had been performed in relatively small chambers under laboratory conditions. None of the experimental data used chlorine dioxide to sterilize a large area.

2.2.3 Generation of Chlorine Dioxide Gas. A two-reaction process designed to minimize the amount of chlorine gas present in the final product was used to generate the chlorine dioxide. A 15 percent hydrochloric acid (HCl) solution was combined with a sodium hypochlorite (NaOCl)

Figure 1. Chlorine Dioxide Generation System. *The chlorine dioxide generation system used by Sabre Oxidation Technologies, Inc. for the onsite generation of the gas.*

solution with between 5 percent and 16 percent available chlorine. This first reaction generated a controlled amount of chlorine gas that reacted with a 25 percent sodium chlorite ($NaClO_2^-$) solution to form a pure ClO_2 gas. Water was pumped through piping above the reaction columns generating negative pressure in the piping resulting in the chlorine dioxide gas partitioning into the water. The chlorine dioxide containing water was pumped into a storage tank. The resulting concentration of the gas in water was approximately 3,000 parts per million volume (ppmv). Air was bubbled through this storage tank to release the dissolved chlorine dioxide gas into the vapor phase. The gas was then pumped into the trailer to reach the desired concentration.

2.2.4 Measuring the Effectiveness of Chlorine Dioxide Fumigation. Methods for measuring effectiveness in eliminating the target contaminant are required for any remediation technology. For example, for a steam autoclave used to sterilize medical equipment, commercially available paper strips bearing dried *Bacillus* spore (that is, "spore strips") are used to measure whether a particular autoclave achieves full sterilization. Spore strips have also been used for many years to monitor the effectiveness of chamber or clean-room fumigations using the gases and vapors listed in Table 1.

Spore strips are small filter paper strips that are impregnated with a fixed number of nonpathogenic *Bacillus* spores and are then placed into a glassine or Tyvek® sealed envelope. The type of spore strip most frequently used contains 10^6 *B. subtilis* organisms. Killing efficiencies are reported in terms of "log kill." A one-log kill means that if 10 organisms are

Figure 2. Chlorine Dioxide Remediation Test Trailer at the Brentwood P&DC. *The cylinder in front is designed to eliminate excess chlorine dioxide and prevent its release into the atmosphere.*

exposed to the treatment, then all are killed. A six-log (10^6) kill means that if one million organisms are exposed to the treatment, then all are killed (99.9999 percent effective killing). A 10^6 killing efficiency is considered by the Food and Drug Administration (FDA) to be sufficient to "sterilize" medical equipment. USEPA uses the same standard of 10^6 kill when registering chemical sterilants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

"Steri-chart" multi-packs are also available that contain spore strips impregnated with 10^4, 10^5,10^6, 10^7, and 10^8 *B. subtilis* organisms. Steri-charts were also obtained for *B. stearothermophilus* impregnated with 10^3, 10^4,10^5, 10^6, and 10^7 organisms. *B. stearothermophilus* is considered a hardier spore for thermal sterilization techniques; therefore, the number of organisms for the Steri-charts is one log less. Using the multi-packs, one can determine the log kill range from less than 10^4 up to 10^8 kill. The log kill is determined by the highest concentration of bacterial spores that yield no growth in a biological assay.

It was clear from the literature that fumigation conditions, including concentration of ClO_2 gas, temperature, relative humidity, and contact time were important parameters for achieving effective killing of the spores. USEPA testing in a trailer at the USPS Brentwood P&DC included monitoring chlorine dioxide concentrations, temperature, and relative humidity measurements, as well as information on survival of surrogate bacterial spore species. "Spore strips" composed of strips of filter paper impregnated with spores from benign *Bacillus* species (*B. subtilis, B. thuringiensis, and B. stearothermophilus*) were used in these tests as surrogates for anthrax spores to determine appropriate ClO_2 gas concentration, temperature and humidity needed to achieve effective remediation.

In accordance with standards utilized by the USAMRIID at Fort Detrick Maryland, a fumigation is considered effective if the spore indicator strips containing 10^6 spores and placed at a frequency of at least 1 per 100 square feet are negative for culture growth after 24, 48, and 72 hours at the appropriate growth conditions. The USEPA team used these same requirements

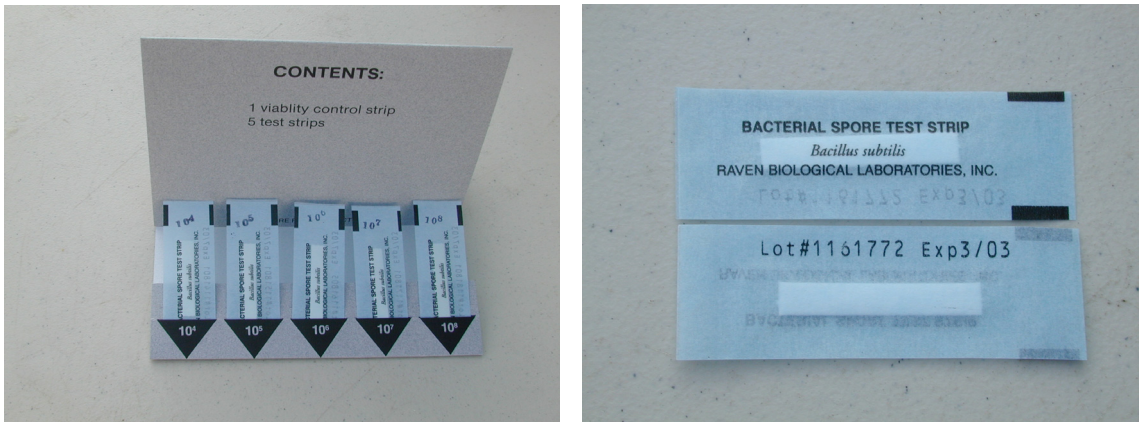


Figure 3. Spore Strips and Steri-chart. *Individual spore strips contain either B. subtilis or B. stearothermophilus.*

to measure the effectiveness of fumigation with chlorine dioxide gas. Specifically, spore strips were used to determine whether the target environmental parameters necessary for effective spore kill (that is, temperature, humidity, gas concentration and total contact time) had been achieved and whether the fumigation was successful.

2.2.5 Protocol for Spore Strip Labeling and Placement. Prior to ClO_2 fumigation testing at the Brentwood P&DC, START personnel placed spore strips containing various *Bacillus* species in glassine or Tyvek envelopes in contaminated areas. The spore strips were used as indicators of the effectiveness of spore kill at various locations in affected areas. The protocol for spore strip labeling and placement in the test trailer is provided as *Appendix 2*. Two individuals worked as a team, with one placing the spore strips while the other was responsible for labeling. Strips were placed in the trailer immediately prior to the start of a chlorine dioxide fumigation run and were removed when the chlorine dioxide levels in the truck fell to below 100 parts per billion (ppb). Approximately six to eight hours were required for the chlorine dioxide to fall to these levels.

The subject of this report is the analysis of conditions and their impact on the effectiveness of chlorine dioxide in Capitol Hill-related remediation efforts. During all test runs in the Brentwood P&DC trailer test, air samples were taken from various ports drilled into the sides of the trailer in order to quantify the levels of chlorine dioxide gas. It quickly became apparent that frequent monitoring of chlorine dioxide levels was critical because the rate of decay of the gas in the trailer was greater than anticipated. Initial runs (test runs 1 through 6) were conducted without internal monitoring of gas levels or internal control of temperature and humidity, and are therefore not subject to further analysis in this report. These factors were controlled in tests 7 through 12 and the data from those tests are included in the analysis.

3. ANALYSIS AND RESULTS

3.1 Brentwood P&DC Trailer Fumigation Analysis

During test runs 1 through 6 the temperature and relative humidity were not regulated and recorded; therefore, they are not subject to analysis. Without accurately knowing these key parameters, graphical analysis is impossible.

Three indicator spore-forming organisms were used for evaluation of sporicidal activity of the chlorine dioxide gas. Results are presented as three-dimensional (3-D) bar graphs. The x-axis represents the total chlorine dioxide concentration over time (CT). The y-axis represents the log kill obtained for that particular organism on a particular run. The z-axis represents the relative humidity. Each run is illustrated as a separate bar whose location is determined by the three graphed parameters. Temperature is another important element and it is graphically illustrated by color. The color key is at the bottom of each graph. The data are also presented in tabular form.

The analysis of the data generated at the Brentwood P&DC trailer facility are complicated by two major observations. The first is that the same number and type of spore strips were not used for every run. Operational parameters and availability, as well as a constantly changing environment, led to inconsistency in spore strip availability for remediation validation. The second observation is more confounding. In run eleven, the Steri-charts for *B. subtilis* and

B. stearothermophilus were completely negative indicating a 10^8 and 10^7 kill, respectively. However, the analysis of individual spore strips containing 10^6 spores each demonstrated 20 percent and 30 percent positive growth, respectively. In this instance, the Steri-chart data validated a high degree of effectiveness of killing. However, individual spore strips from a different lot number that contained a fixed number of spores generated conflicting data. The Steri-chart data have been graphically illustrated, but this creates doubt about the validity of spore strip analysis.

Three Brentwood P&DC test trailer 3-D bar graphs were generated illustrating five factors: relative humidity, temperature, concentration of chlorine dioxide integrated over time (CT), log kill rate, and organism type. Figure 4, *B. subtilis,* Figure 5, *B. stearothermophilus,* and Figure 6, *B. thuringienisis,* graph kill rate, concentration of chlorine dioxide integrated over time (CT), and relative humidity (%RH) on three axes with temperature color coded. Temperatures are color coded in incremental ranges from less than 75.1°F (blue) to greater than 85°F (red) with a series of colors within. The organisms are identified by different patterns on the bars of the graphs: *B. subtilis* has vertical lines, *B. stearothermophilus* has horizontal lines, and *B. thuringienisis* has no pattern. The same scaling was used on the axes to allow for easier comparison.

Relative Humidity Data. Data were taken from Table 2. Test runs 1 through 6 were not used as part of this data set since temperature and humidity readings were not monitored inside the trailer until Test 7. Relative humidity data was taken from Actual Average RH (%).

Temperature Data. The data were taken from Table 2. Test runs 1 through 6 were not used as part of this data set since temperature and humidity readings were not monitored inside the trailer until Test 7. Temperature data was taken from the Actual Average Temp (°F).

Concentration Data. The data were taken from Table 2. Test runs 1 through 6 were not used as part of this data set since temperature and humidity readings were not monitored inside the trailer until Test 7. To calculate concentration values, the number of hours of treatment (12 hours for each test run) was multiplied by the Actual Average Concentration, which was measured in ppmv.

Kill Rate Data. Kill rates were taken from the laboratory analysis data of the indicator spore strips and Steri-charts. If half of the Steri-charts indicated a 10^8 kill and the other half indicated a 10^7 kill, the value calculated was a $10^{7.5}$ kill. The actual analysis results are found in *Appendix 3*.

Table 2 provides summary data from the test runs at the Brentwood P&DC trailer facility. Details of the specific laboratory analyses conducted at Fort Meade, Maryland, and Berkeley, California, are contained in *Appendix 3*.

Run # 7

This run had Steri-charts for both *B. subtilis* and *B. stearothermophilus*. This run represented the lowest CT value, 2400, less than half of the target concentration of 9000. The temperature and humidity were within the range of the other experiments and the killing efficiency was within the acceptable range. This run supports the finding that the experimental parameters for chlorine

Table 2. Summary of ClO_2 Trailer Tests at USPS Brentwood P&DC

	Test 7	Test 8	Test 9	Test 10	Test 11	Test 12, Run #1	Test 12, Run #2
Date	11/03/01	11/06/01	11/08/01	11/10/01	11/13/01	11/26/01	11/27/01
Objective	Materials Test	Equipment test	Equipment test	Bio. kill test	Mail test	To determine the effectiveness of a reapplication of ClO_2 gas	To determine the effectiveness of a reapplication of ClO_2 gas
Time Start	1718 on 11/03	1445 on 11/06	1000 on 11/08	1130 on 11/10	1445 on 11/13	1602 on 11/26	1445 on 11/27
Time End	0520 on 11/04	0245 on 11/07	2200 on 11/08	2330 on 11/10	0245 on 11/14	0400 on 11/27	0245 on 11/28
Target Concentration	500 ppmv 12 hours	500 ppmv 12 hours	1000 ppmv 12 hours	750 ppmv 12 hours	2200 ppmv 12 hours	500 ppmv 12 hours	500 ppmv 12 hours
Actual Average Concentration	200 ppmv	553 ppmv	1002 ppmv	742 ppmv	2364 ppmv	557 ppmv	512 ppmv
Duration	12 hours	12 hours	12 hours	12 hours	12 hours	12 hours	12 hours
Target Temp (°F)	80	70	80	75	75	75	75
Actual Average Temp (°F)	78	75	77	77	77	78	78
Target RH (%)	70	60	70	75	75	75	75
Actual Average RH (%)	71	65	74	79	77	81	81
Ambient Temp (°F)	60	55	65	62	49	60	58.5
Ambient RH (%)	46	35	42	40	N/A	N/A	N/A
BC (total count)[a]	N/A	N/A	N/A	N/A	55	0	0
BM (total count)	N/A	N/A	N/A	N/A	55	0	0
BS (total count)	30	90	80	90	55	0	0
BT (total count)	60	90	80	65	55	0	0
SS (total count)	50	90	80	75	175	100	100
SC-BS (total count)	8	0	0	0	7	12	11
SC-SS (total count)	7	9	11	20	7	14	14
NC (total count)	33	48	48	45	26	12	12
PC (total count)	0	0	0	0	0	N/A	N/A

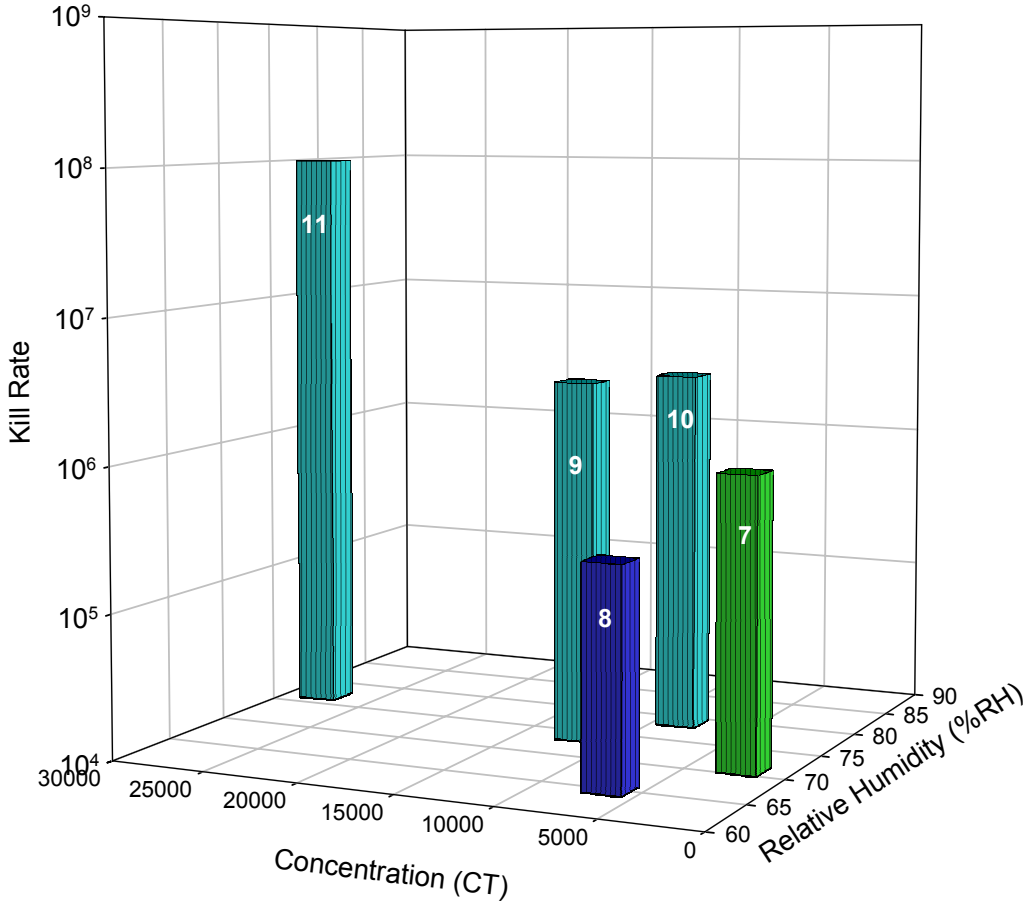
Notes:

[a] Total count means the total number of spore strips, steri-charts, of control strips used in the test.

BC = *B. cereus* spore strip
BM = *B. megaterium* spore strip
BS = *B. stearothermophilus* spore strip
BT = *B. thuringiensis* spore strip
SS = *B. subtilis* spore strip
ClO_2 = Chlorine Dioxide
N/A = Not Applicable
Ppm = Parts per million volume
SC-BS = *B. stearothermophilus* steri-chart
SC-SS = *B. subtilis* steri-chart
NC = Negative control
PC = Positive control

Source: "Chlorine Dioxide (ClO_2) Gas Phase Demonstration Project" by the United States Environmental Protection Agency, Environmental Response Team (ERT).

Brentwood P&DC Test Trailer- *B. subtilis*
RH vs. CT vs. Kill Rate



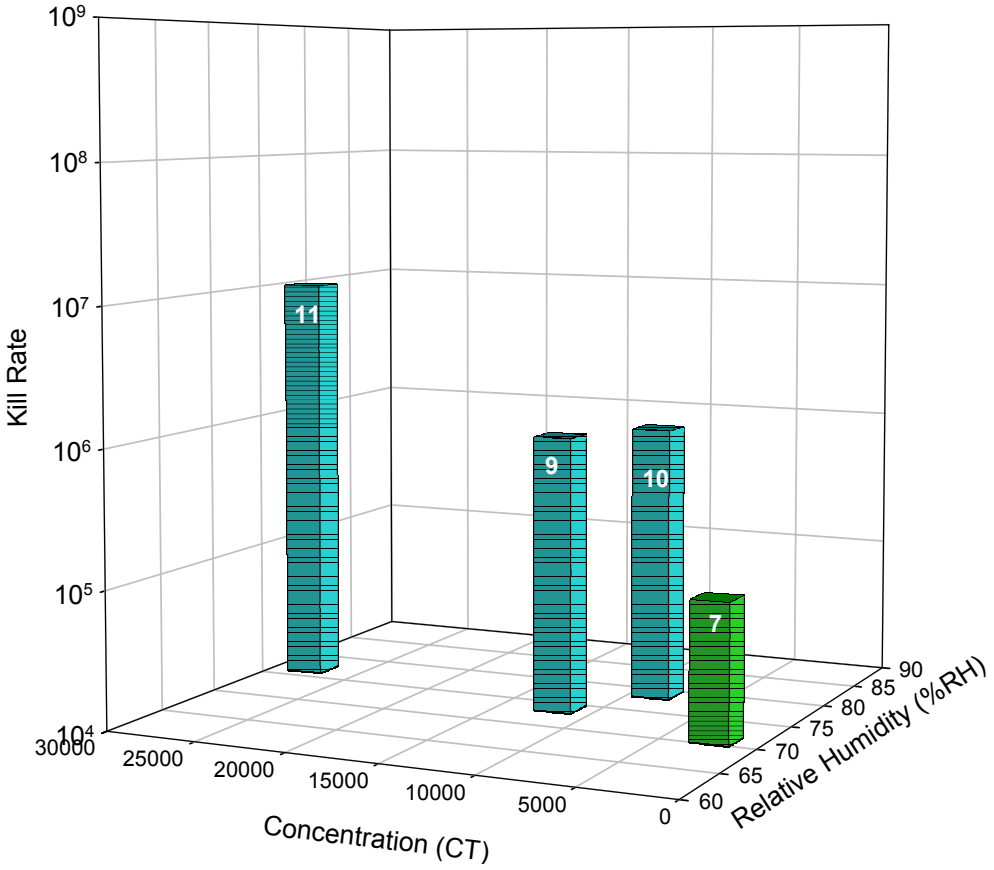
Brentwood P&DC Test Trailer—*B. subtilis*

Test Run	%RH	Temp (°F)	CT	Kill Rate
7	71	78	2400	10^6 Kill
8	65	75	6600	$10^{5.5}$ Kill
9	74	77	12000	$10^{6.5}$ Kill
10	79	77	8700	$10^{6.5}$ Kill
11	77	77	28000	10^8 Kill

Temp Range (°F)	Color Key
<75.1	
75.1-77.5	
77.6-80.0	
80.1-82.5	
82.6-85.0	
>85.0	

Figure 4. Brentwood P&DC Test Trailer—B. subtilis. *B. subtilis killing activity during test runs 7 through 11 at the Brentwood P&DC test trailer.*

Brentwood P&DC Test Trailer- *B. stearothermophilus*
RH vs. CT vs. Kill Rate



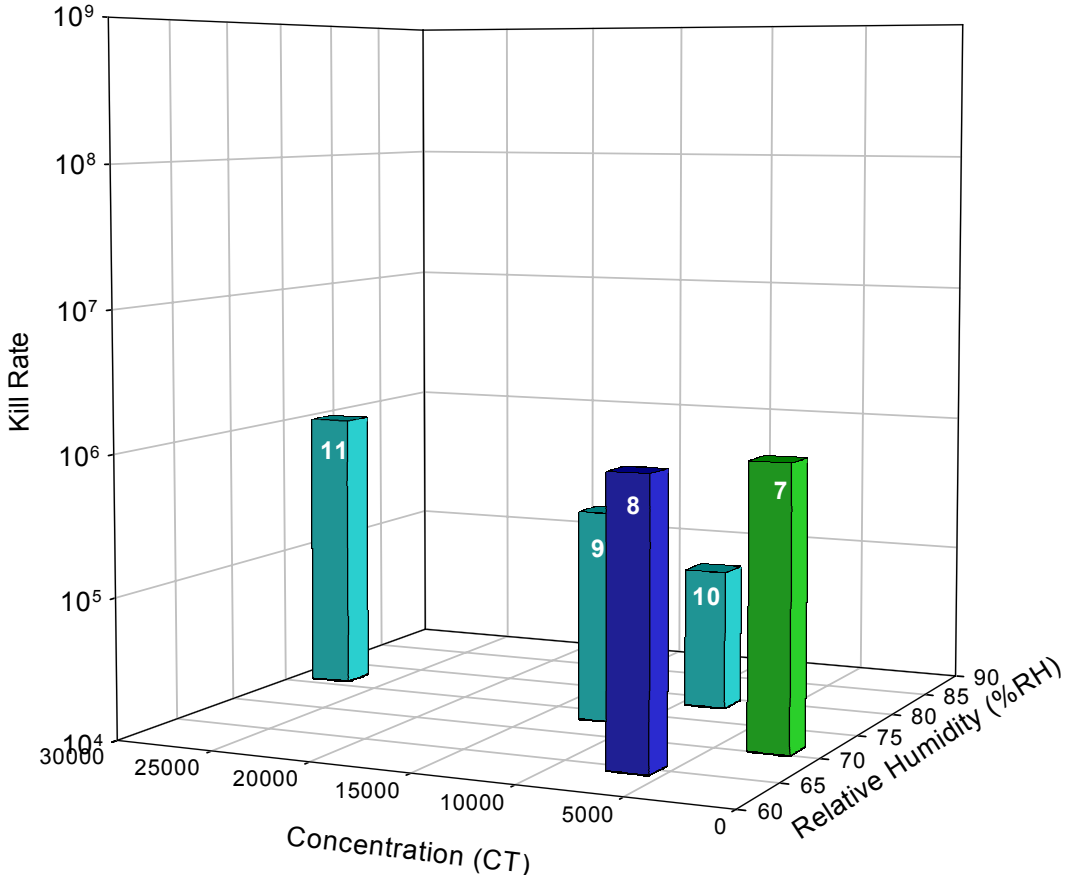
Brentwood P&DC Test Trailer—*B. stearothermophilus*

Test Run	%RH	Temp (°F)	CT	Kill Rate
7	71	78	2400	10^5 Kill
8	65	75	6600	N/A
9	74	77	12000	10^6 Kill
10	79	77	8700	10^6 Kill
11	77	77	28000	10^7 Kill

Temp Range (°F)	Color Key
<75.1	
75.1-77.5	
77.6-80.0	
80.1-82.5	
82.6-85.0	
>85.0	

Figure 5. Brentwood P&DC Test Trailer—B. stearothermophilus. *B. stearothermophilus killing activity during test runs 7 through 11 at the Brentwood P&DC test trailer.*

Brentwood P&DC Test Trailer- *B. thuringienisis*
RH vs. CT vs. Kill Rate



Brentwood P&DC Test Trailer—*B. thuringienisis*

Test Run	%RH	Temp (°F)	CT	Kill Rate
7	71	78	2400	10^6 Kill
8	65	75	6600	10^6 Kill
9	74	77	12000	$10^{5.5}$ Kill
10	79	77	8700	10^5 Kill
11	77	77	28000	10^6 Kill

Temp Range (°F)	Color Key
<75.1	
75.1-77.5	
77.6-80.0	
80.1-82.5	
82.6-85.0	
>85.0	

Figure 6. Brentwood P&DC Test Trailer—B. thuringienisis. *B. thuringienisis killing activity during test runs 7 through 11 at the Brentwood P&DC test trailer.*

dioxide remediation have not been suitably defined. The technology is quite effective, but the optimal parameters have not yet been determined. Although the CT value is low, the relative humidity was slightly above 70 percent, possibly contributing to a more effective kill than originally anticipated.

Run #8

This run had a higher CT value than run 7, but the relative humidity was low. Although the *B. thuringiensis* kill rate was acceptable, the *B. subtilis* kill rate was decreased. The *B. stearothermophilus* data was not graphed because 64 of the 70 test strips were positive for growth. This run points out some valuable information. It indicates that relative humidity is critically important. These data are consistent for a higher requirement for relative humidity. It also points out the value of *B. stearothermophilus* as an indicator organism. That is, the fact that this organism is cultured at 60°C minimizes the chance of accidental contamination of the spore strip during handling.

Run #9

Run 9 had twice the CT as compared with run 8 with higher humidity. The *B. thuringiensis* killing efficiency appears unchanged from run 8 while the other two indicator organisms had increased killing efficiency, supporting the requirement for relative humidity levels in excess of 70 percent.

Run #10

This run came closest to generating the operational parameters targeted during the HSOB fumigations. Run 10 was similar to the results found for run 8 in that many (38 of 60) of the *B. thuringiensis* spore strips yielded positive results. However, the results for both *B. subtilis* and *B. stearothermophilus* indicated the target level of kill, 10^6. Steri-charts were only available for *B. subtilis* and they were in agreement with the single spore strips containing 10^6 spores. This run provided the basis of target fumigation parameters for subsequent fumigations in the HSOB.

Run #11

Judging from all of the earlier runs, one would predict that the kill levels for this run would be in excess of all the previous runs due to the elevated temperature, humidity, and CT values. In fact, the graph indicates this is true. The graph was based on the Steri-chart results that indicated no positive growth (7 tests) for *B. subtilis* at 10^8 organisms and no positive growth for *B. stearothermophilus* (4 tests) at 10^7 organisms. Individual spore strips containing 10^6 organisms for each organism were assayed at the two reference laboratories. The Berkeley laboratory reported 28 of 155 as positive for *B. subtilis*, and 10 of 35 as positive for *B. stearothermophilus*. The Fort Meade reference laboratory reported 1 of 20 as positive for *B. subtilis*, and 0 of 19 positive for *B. stearothermophilus*.

The results of these preliminary tests and subsequent analysis indicated that the target concentration of ClO_2 gas should be 750 ppmv for at least 12 hours yielding a final CT of 9,000. Tests confirmed that temperature and humidity were critical parameters and that they should be

kept at a minimum of 75°F, and 75 percent RH, respectively, to enhance kill rather than increasing the concentration of ClO_2 gas.

3.1.1 Materials Compatibility Studies. Various materials were included in the trailer during numerous runs at the Washington DC test facility. The results are subjective, but present a good overview of the susceptibility of various materials to the oxidizing potential of chlorine dioxide, and are summarized in Table 3.

Effect of Chlorine Dioxide on Various Materials. Materials exposed to an average of approximately 9000 ppmv over a 10-hour period.

Table 3. Material Summary

Item	Effect of ClO_2 Treatment
Gold taffeta fabric	Spotting, bleaching
Congressional document	Spotting, slight bleaching
B/W photo	Bleached
Photo negative	None
Artwork Kodak Digital A dye	None
9A Fuji pictorio paper	Faded and bleached
8A Fuji pictorio paper	None
Purple cotton fabric	Bleached
Negative SA silver Kodak gray scale	None
Black leather	Faded
Dark brown felt	Faded
Pink wool carpet	None
Baleen	None
Wooden plaque with brass	None
Ivory scrimshaw pendant	None
5 pennies	Shiny on exposed side
Brass knob	None
Peach colored fabric	Bleached
Purple velvet	Badly bleached
Peach furniture fabric	None
Nalgahyde	None
Color photo (Capitol Bldg.)	Bleached, spots
B/W drawing and photo in wood frame with fabric matt	None
White marble slab	None
Red marble slab	None
Wallboard	None

3.1.2 Use of Steri-Charts for Analysis. The testing at the Brentwood P&DC trailer represented the first field-testing of the sporicidal activities of chlorine dioxide. These tests provided valuable data for the ensuing remediations of the HSOB as well as later tests at the Beltsville, Maryland, trailer location.

The spore strips used in the Brentwood P&DC test facility were sent to two separate laboratories for analysis. The first was to the laboratory of Dr. Terrence Leighton at University of California (UC), Berkeley. The second laboratory was the USEPA reference laboratory at Fort George Meade, Maryland. Dr. Leighton is one of the premier U.S. scientists working on *B. anthracis* spores and also conducts research on chlorine dioxide effectiveness for killing bacterial spores.

The Berkeley laboratory results for fixed concentrations of spores are frequently in conflict with the Steri-charts, while the Fort Meade results support the Steri-chart data. The Berkeley laboratory assayed Steri-charts for both organisms while the Fort Meade only assayed Steri-charts for *B. subtilis*. There are insufficient data to draw meaningful conclusions between the two sets of data.

It is apparent by the data represented here that the use of Steri-charts presents both an advantage as well as a challenge. The advantage is that one can determine log kills over a much wider range than is possible with a single concentration of organisms. The challenge is that without confirmatory cultures to verify whether the indicator organism or a contaminant in the system is responsible for growth, the conflicting Steri-chart data are questionable.

3.2 Selection of Laboratory for Analysis of Spore Strips from HSOB

As time progressed and the conditions for effective killing of the spores were established, the team realized that spore strips placed in contaminated regions of the HSOB could potentially be contaminated with viable *B. anthracis* spores from the anthrax-laden letter, even after chlorine dioxide treatment. Because of this risk, it was determined that the laboratories performing spore strip processing and analysis activities must be certified by the CDC to have Biosafety level 3 (BSL-3) facilities in which the spore strips would be processed. At that time, UC Berkeley was unwilling to accept material potentially contaminated with *B. anthracis* spores, and the CDC had not yet certified the USEPA BSL-3 facility at Fort Meade. Thus, another laboratory had to be identified to process and analyze the spore strips.

Dr. Leighton previously worked with researchers at the Southwest Research Institute® (SwRI®) and knew that they had BSL-3 facilities in which to conduct the analysis. He contacted Dr. Mike McNaughton at SwRI® who stated that SwRI® was interested in conducting the analysis. SwRI® proposed a competitive flat rate fee for each spore strip that was determined competitive and contract initiation began with USEPA Region III.

SwRI®, headquartered in San Antonio, Texas, is a multidisciplinary, independent, nonprofit, applied engineering and physical sciences research and development organization with 11 technical divisions. These technical divisions span the areas of physics, materials sciences, nuclear waste, space, chemistry and engineering. There is no technical area highlighted that specializes in biology or life sciences. However, SwRI®'s Chemistry and Chemical Engineering Division has provided support to the USEPA's ERT for many years in the analysis of

environmental samples. Approximately 50 percent of their service contracts support USEPA Super Fund analysis and analytical tests are generally for chemical and nuclear waste testing.

Within the Chemistry and Chemical Engineering Division, the spore strip analyses were conducted by the Environmental Engineering Section of the Environmental and Demilitarization Technology Department. Prior to the Capitol Hill incident, the Chemistry and Chemical Engineering Division's experience with analysis of biological samples was extremely limited. Of the 270 presentations listed for the department on the SwRI® Web site, only two involved microorganisms. The focus of these presentations was modeling for predicting dispersion in water and aerosol monitoring for microorganisms. The Environmental Engineering Section had not previously conducted spore strip analysis for the USEPA, but at least one senior individual in the Chemistry and Chemical Engineering Division had previous laboratory experience with spore strip analysis. In addition, SwRI® had adequate facilities to perform the analysis, including a BSL-3 laboratory, and they were an ISO 9002 certified reference laboratory and used all proper procedures and tracking mechanisms for chain of custody handling and analysis of samples.

The BSL-3 laboratory facility where the analyses were conducted is a single room approximately 150 to 200 square feet in size. There is a sink, a workbench, and a 6-foot sterile hood with two laboratory chairs. Although the facility is austere, it is sufficient in size and equipment for the task undertaken. In addition, the researchers at SwRI® arranged to collaborate with a sister institute, Southwest Foundation for Medical Research, located within one mile of their facility. Initial plans were to split samples between the two institutes; however, this did not occur due to the timing of the need.

Communications were established between the laboratories at UC Berkeley and SwRI®. A spore strip processing and analysis protocol from UC Berkeley (*Appendix 4*) was passed to SwRI® where they followed the procedure using pristine spore strips to determine compatibility with their facilities. Modifications were made by SwRI® and documented in their SOPs. These revisions were cleared with USEPA and Dr. Leighton. Although SwRI® scientists developed a protocol for the pristine spore strips, they never conducted test runs with spore strips exposed to chlorine dioxide using procedures and under conditions used in the HSOB. This is a key observation because the conditions of the spore strips sent to SwRI® from the HSOB for analysis were significantly different than the pristine spore strips.

3.3 Chlorine Dioxide Fumigation Activities

The first ClO_2 fumigation of the offices where the contaminated letter was delivered in the HSOB was conducted between November 30, 2001, and December 2, 2001. Spore strips were placed in the suite on November 30, 2001, by four- or five-person teams dressed in Level C PPE. The teams placed spore strips on surfaces including desktops, walls, cabinets, drawer interiors, floors, plenums, and mail slots. They attached the folders containing the spore strips using packing tape, staples, or pushpins. The methodologies used to anchor the spore strips to the various locations became problematic for the laboratory personnel conducting the analysis.

Steam was introduced into the suite until the humidity and temperature reached target levels. Aqueous ClO_2 gas was generated outside of the building and piped inside where it passed through an air stripper and was delivered into the suite in gaseous form. Circulating fans were used to distribute the ClO_2 gas evenly. Temperature, humidity, and ClO_2 gas concentration were

monitored and adjusted as needed throughout the exposure period of 12 hours. Air samples were collected every 10 to 15 minutes from numerous locations. The ClO_2 gas concentration inside the building was calculated by running air through an impinger containing 15 milliliters of a strong potassium iodide buffer and a gram of iodide crystals for 3 to 5 minutes at 1 to 2 liters per minute. The sample was added to 150 milliliters of distilled water and titrated with sodium thiosulfate to calculate the concentration of ClO_2 gas.

At the end of 12 hours, the ClO_2 gas was shut off and a caustic bisulfite solution was piped into the suite to react with the residual ClO_2 gas and destroy it, a process referred to as "scrubbing." In addition, ClO_2 dissipates rapidly and decays under normal environmental conditions. To ensure the safety of the surrounding community, USEPA monitored the concentration of ClO_2 outside of the building during all fumigations using the trace air gas analyzer (TAGA) mobile laboratory, which is capable of detecting ClO_2 in the parts per trillion range. Monitoring indicated that the level of ClO_2 gas outside the building never exceeded 25 ppb.

The sampling teams retrieved the spore strips on December 3, 2001. The sample folders were collected and a clean sheet of paper was put inside each one to avoid cross-contamination between spore strips and Steri-charts on opposing sides of each folder. Each folder was sealed inside a 2-gallon Ziploc® bag and grouped into a legal-size accordion folder with other folders from the same area of the suite. The accordion folders were passed through a decontamination chamber into a 3-gallon Ziploc® bag held by a team member in the contamination reduction zone. The bag was then passed through a portal to the exterior of the building where it was inserted into another 3-gallon bag and sealed. START personnel had previously prepared chain of custody paperwork containing a unique identifier for each sample folder and compared them to samples retrieved from the building.

Figure 7. Chlorine Dioxide Generation System for HSOB. *The onsite chlorine dioxide generation system for all HSOB operations.*

Figure 8. Interior Operations During the Office Remediation Operations. *Note the tubing and other obstacles on the floor. This area is outside the actual fumigation area that had even more obstacles.*

Although the spores impregnated on the spore strips were benign, they were placed in an environment known to be contaminated with anthrax spores. Thus, they were considered potentially infectious and needed to be handled and shipped using special procedures. The samples were packaged and shipped in compliance with International Air Transport Association (IATA) Dangerous Goods Regulations to SwRI® where they were processed and analyzed in a BSL-3 laboratory.

3.3.1 Spore Strip Placement and Retrieval Training. The combination of scope, immediacy, and novelty of the spore strip placement and retrieval process undoubtedly contributed to confusion and some level of cross-contamination. Initial spore handling procedures at the Washington, DC, trailer location indicated a lack of experience handling media of this type. The beauty of this type of analysis is also a difficulty, if one viable bacteria or spore is present on the strip, the culture will demonstrate a positive signal.

USEPA ERT teams contacted Dr. Leighton, UC Berkeley, who provided a spore handling protocol (*Appendix 5*). Standardization of the spore handling protocol resulted in significantly more uniform results than at the Brentwood P&DC test trailer. Because the magnitude of test strips, hundreds in the trailer and thousands in the office complex, a new team was trained. Although the team was motivated, no training documentation is available. The first time these people placed and removed spore strips was for the actual fumigation. Those individuals who had hands-on experience with spore strips in the trailer served as supervisors.

This clearly was not an optimal means of operation. However, it must be stressed that time was a critical factor, and individuals still did not know what the key operational parameters were; therefore, they could not be adequately controlled. The spore strips were sealed in translucent envelopes and appeared isolated from the remaining environment. Individuals conducting the placement and collection did not have documented training in aseptic technique.

Spore placement, removal, and shipping were not the only opportunities to introduce contamination into the system. The other end of the operational system was the analytical laboratory. Analysis of the spore strips from both the office and HVAC remediation operations was SwRI® laboratories in San Antonio, Texas.

3.4 HSOB Fumigation Operations

Over 3,000 verification spore strips were placed in various locations to measure the effectiveness of the fumigation. The Excel spreadsheet indicating the placements of the strips is presented in *Appendix 6*. Spores were placed on walls, floors, desks, tables, under desks. The multiple areas for spore strip placement were to assure that the gas reached all areas within the suite and that an effective kill level was achieved. Spores were placed in regions of known contamination as well as areas where contamination was not identified. Since the chlorine dioxide is a gas, fans were located in strategic locations on floors 5 and 6 to facilitate the distribution of the gas. Eight box-type fans were placed on the fifth floor and one was placed on the sixth floor. In addition there were six heaters and humidifiers placed on each of the two floors to reach and maintain proper temperature and relative humidity (*Appendix 7*).

Temperature and humidity measurements were initially to be conducted in multiple locations on both floors. Due to an electrical problem, these monitoring devices were rendered inoperable the night of operations. The solution was to send individuals into the operations areas during various times of the fumigation to take measurements on floors 5 and 6. The Excel spreadsheet of the recorded temperature and relative humidity measurements is found in *Appendix 8*. Temperatures on the fifth floor were relatively constant around 73 to 74°F. Temperatures on the sixth floor ranged from 72 to 77°F. Relative humidity measurements were very constant on the fifth floor ranging from 83 to 89 percent. The values on the sixth floor were much more variable and ranged from a low of 57 percent to a high of 75 percent. The relative humidity values on the sixth floor did not maintain the target value of 75 percent. Prior and subsequent testing of chlorine dioxide efficiency in killing spores suggests a less effective remediation on the sixth floor. This is puzzling since the killing efficiency was actually better on the sixth floor relative to the fifth floor.

Chlorine dioxide measurements were conducted at eight separate locations on each of the two floors. The actual CT readings and the locations of sampling stations are found in *Appendix 9*. The gas samples were titrated for chlorine dioxide levels onsite. Total chlorine dioxide levels (CT) on the fifth floor ranged from 7,477 to 11,674 ppmv with an average of 9,641 ppmv. Levels on the sixth floor were more variable with a low of 5,887 ppmv and a high of 17,723 ppmv and averaging 10,895 ppmv.

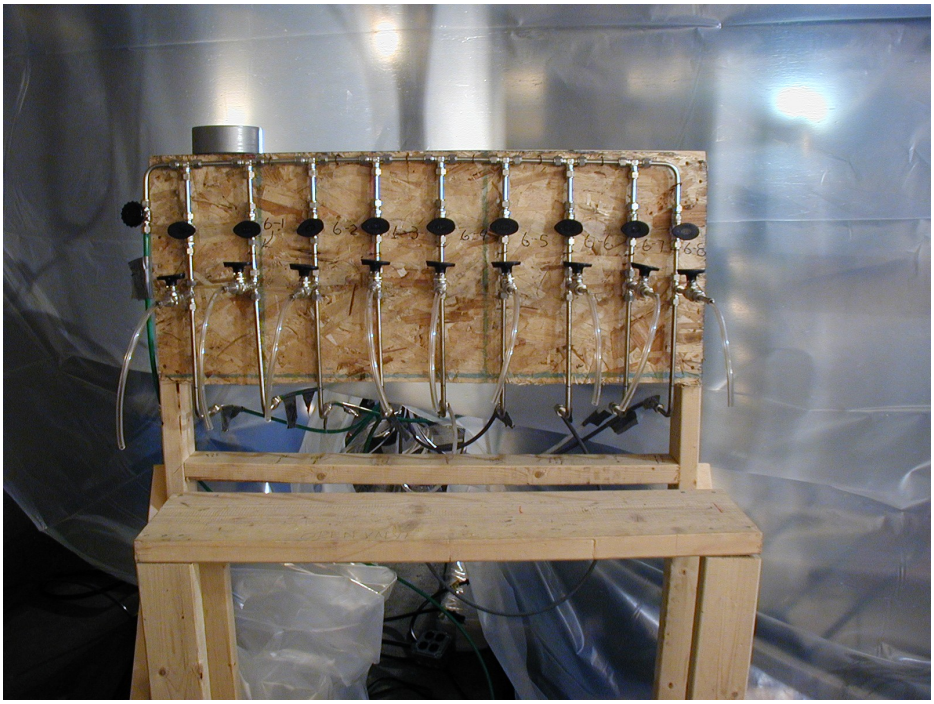


Figure 9. Sampling Manifold for Measurement of Chlorine Dioxide Concentrations from Eight Different Locations on the Sixth Floor. *An identical apparatus was constructed for operations on the fifth floor.*

3.4.1 Spore Strip Sample Processing. An overriding theme throughout the Capitol Hill Incident was the constant temporal pressure. This pressure was present at all levels of the response and was compounded by the fact that no such response had been conducted from which to learn. Although good scientific principles were the driving factor for everyone, the pressure to perform well and in a timely fashion was on everyone's mind. The first samples provided to SwRI® for analysis were those from the Daschle suite within the HSOB. SwRI® was required to process over 3,000 samples in one day with very little ramp up time. The tracking and handling requirements were considerable. As mentioned previously, SwRI® had ISO 9002 certified practices and personnel in place to handle chain of custody requirements associated with processing the samples, but the volume and compressed timeframe presented a real challenge. In addition, some samples were missing and a few were damaged before arriving at SwRI®. All of these secondary issues were clearly documented by the SwRI® scientists. Four SwRI® employees, working in teams of two, processed the first set of 3,146 samples within 25 hours of receipt. While this large volume for a small staff is not considered to be optimal, the requirement was to process the samples within a 24-hour period.

The spore strip samples arrived at SwRI® via FedEx shipping. The packaging of the samples was adequate, but many of the individual spore strips and the Steri-charts were difficult to open as they were taped on both ends when positioned in the HSOB. The methods used to secure the spore strips within the HSOB left them in a condition that prevented the use of the "Spore Strip Transfer Protocol-Leighton Labs." Specifically, both ends of the glassine envelopes containing the spore strips were taped to the folder in many instances. This meant that the glassine envelopes be cut with a scalpel, then squeezed to expose the spore strip and removed with

flamed tweezers. In addition, the chlorine dioxide and steam treatment appeared to "bond" the side of the glassine envelopes together making separation difficult. The need to cut the glassine envelope and the difficulty in obtaining separation increased the potential for contamination of the spore strips during the transfer to the growth media. This was confounded by the fact that all packaging was opened in the hood of the SwRI® BSL-3 facility where two persons were working simultaneously in protective clothing. It must be emphasized that the quantity of packaging material that had to be manipulated within the biological safety cabinet to reach the spore strip samples was voluminous.

The quantity of samples in each shipment typically numbered between 250 and 450 samples each. Each individual spore strip was inside a key envelope, and typically 10 to 18 envelopes were batched inside a plastic bag, which then was enclosed in one or two more plastic bags. Only the exterior of the outer-most bag was presumed to be free of *B. anthracis*. Therefore, the removal of the spore strip samples from the packaging had to be performed in the biological safety cabinet inside the BSL-3 laboratory.

The first four boxes of spore strips from the HSOB arrived at SwRI® intact at 0930 on Tuesday, December 4, 2001. The Chain of Custody (COC) sheets had been previously faxed to SwRI® on Sunday, December 1, 2001, and the sample data transferred to labels for the growth media tubes. The transfer of the spore strips from the sample folders to the growth media tubes began at approximately 1230 in the BSL-3 laboratory. The transfer of the 3,146 spore strips to the tubes and then into the incubator took approximately 25 hours. The first data recording (observing each tube for color change) was at 0900 Thursday, December 6, 2001 (Day 1). The condition of each tube was recorded as a – (purple), +/- (intermediate between purple and yellow) and + (yellow). This was repeated each morning for the required 7 days. Growth data were entered into the spreadsheet and e-mailed to USEPA. USEPA was kept informed of progress and the posting of the data by phone. The data were posted on a secure Web site for access by USEPA, which proved to be the easiest method for the USEPA to access the data on a real-time basis.

Determinations for the presence of viable bacterial spores on the indicator spore strips were conducted to measure the efficiency of kill for each remediation operation using the chlorine dioxide gas sterilization technique. The spore strips were not appropriate for evaluation of the killing efficiency of liquid or foam decontamination methods. The protocol for culturing of the spore strips is found in *Appendix 10*. The trypticase soy broth (TSB) media vials used to incubate the spore strips contain a pH indicator that changes color (purple to yellow) when microbial growth occurs (that is, metabolic products from the organisms include various organic acids that causes the growth media to become slightly acidic). The original spore strip analysis protocol specified the following classification score for the vials (+) = growth (yellow), (-) = no growth (purple), (+/-) = intermediate growth (any color in between yellow and purple).

The original protocol received stated the spore strips should be incubated at 37°C. After discussion with Dr. Leighton it was evident that the *B. stearothermophilus* spore strips should have been incubated at 60°C. Dr. Leighton sent a revised protocol that included the separate 60°C incubation temperature for the *B. stearothermophilus*. Since the tubes had already been incubated at 37°C for 2 days before being transferred to the 60°C incubator, day 3 is really day 1 in the data table. This error causes a degree of uncertainty in the data for this organism. The advantage of using *B. stearothermophilus* is that it requires an elevated temperature for optimal growth. Most bacteria do not grow well at this higher temperature, and therefore the likelihood

of an external contaminant growing in the culture tube is greatly diminished. This is particularly important in light of the fact that many of the bacteria that grew in the tubes appear not to be the bacteria inoculated in the test spore strips.

The SwRI® laboratory continually sought and instituted a series of practices to eliminate contamination introduced inside the biological safety cabinet while still retaining the ability to culture the spore strips within one to two hours of receipt at the laboratory. In addition to the ethanol spraying of gloves and work surfaces during the spore strip transfer process and frequent glove changes, the procedures described in the following paragraph were implemented during the course of the effort.

Because of the temporal pressure to provide results with regard to the effectiveness of remediation actions, the spore strips were processed immediately after they were taken out of the layers of packaging. During the spore strip transfer process, each person processing samples maintained a log to document any abnormalities that occurred or were observed during the process. Typical incidents included: a spore strip flipping out of its glassine or Tyvek envelope and falling onto the bench of the biological safety cabinet (such strips were always picked up using tweezers and then inserted into the media vial), a media vial cap dropping onto the bench while attempting to open/close the cap, inadvertent contact between a gloved hand and the spore strip or the top of the media vial, or torn/damaged glassine/Tyvek envelopes. All of these annotations were transferred onto the Excel spreadsheets presenting the culture results. Such incidents, which would logically appear to possess a high risk of contamination rarely yielded positive culture results. The logic is that these samples would be expected to contain a higher incidence of bacterial growth due to mishandling errors, especially if the hood had been contaminated by packaging material. Since an increase in bacterial growth was not observed in these samples, the SwRI® scientists argued that the technique used to handle the spore strips was not the cause of the contamination.

SwRI® scientists proposed that a potential source of contamination responsible for the elevated frequency of false positives was the multiple layers of packaging containers (plastic Ziploc bags and small "key" envelopes) used to safely transport the spore strip samples to the laboratory. Dragging all of this non-sterile packaging material into the biological safety cabinet posed a serious challenge. Laboratory personnel frequently sprayed copious volumes of ethanol on the working surfaces inside the cabinet and their gloves throughout the spore strip transfer process to minimize contamination through opportunistic vegetative organisms. Personnel frequently changed their gloves during the processing of the samples. However, contamination of the packaging materials with spores at some point during the entire sampling process, possibly facilitated by static charges between the plastic Ziploc bags and the spores posed a real challenge to SwRI® control measures. Moreover, ethanol has been shown to be bactericidal but not sporicidal; therefore, it is conceivable that *B.anthracis* spores from the HSOB could have contaminated some of the spore strips.

3.5 Spore Strip Results for the HSOB Daschle Suite Fumigation

The fumigation of Senator Daschle's office in the HSOB represented the single largest remediation with chlorine dioxide gas at that point in time. This team effort was the culmination of several weeks of efforts to identify, characterize, and isolate the regions of highest contamination within the complex office structure. Over 3,000 spore strips were placed in

various locations within the remediation site along with temperature and humidity monitors and collection points for chlorine dioxide gas. At the conclusion of remediation, operations teams were dispatched to collect the indicator spore strips. These strips were packaged and sent away for laboratory analysis.

Spore strip culture data were used in combination with data available regarding the temperature, relative humidity, and chlorine dioxide concentrations in the office area of the HSOB. Temperature and relative humidity reached the target values prior to initiation of fumigation. The nature of the office structure posed a significant challenge for even distribution and circulation of air within the office. Even with this obstacle, significant killing of spores was observed in many areas. Some areas demonstrated very poor killing.

In an attempt to correlate spore strip special placement with effectiveness, the bacterial growth data from the office area remediation of the HSOB was subjected to further analysis. The raw data are included in *Appendix 6*. The CD version of *Appendix 6* also contains the Excel file that will allow the reader to manipulate the data using Pivot Tables the authors constructed for a detailed analysis of the data.

Original data from the spore strip analysis from the fumigated rooms were provided in the Excel worksheet, *Hart Results-12-12-01.A*. By combining the data from the file, *Spore Strip Location*, with *Hart Results-12-12-01.A* and modifying the format, a new spreadsheet was created, *HSOB Data Expanded-Pivot*. This new spreadsheet allows for multiple manipulations and representations of the data, including filtering, sorting, PivotTable reports, and PivotChart reports. Six worksheets are in the *HSOB Data Expanded-Pivot* file: *Complete Data, PivotChart-Bact by Key Descr*, *PivotTable-Bact by Key Descr*, *PivotChart-Bact by Gen Loc*, *PivotTable-Bact by Gen Loc*, and *Lookup Tables*. **When opening the file, *HSOB Data Expanded-Pivot (Appendix 6)*, the links should not be updated.**

Worksheet 1. Complete Data. The first worksheet in the *HSOB Data Expanded-Pivot*, *Complete Data*, allows for filtering and sorting by individual parameters such as area (for example, a specific cubicle), key description (for example, back wall), general location (for example, wall), type of bacteria or control (for example, *B. subtilis*), and multiple combinations therein. This worksheet allows the user to easily filter for items such as the negative controls. This data is the basis for the PivotCharts and PivotTable worksheets that follow.

The original data file showed a Sample ID, Lot Number Tube, and seven columns of positive and/or negative indicators (listed from Day 1 to Day 7) that showed bacteria growth following ClO_2 treatment. To allow for better analysis, the Sample ID was broken out into its component parts and coupled with codes taken from the *Spore Strip Location* file. Nine additional columns were created as follows:

1. Floor—two floors were sampled: floor 5 and floor 6.

2. Area—34 separate areas were identified (for example, cubicles identified by numbers, etc.). In a few instances, the samples did not have a counterpart in the *Spore Strip Location* file and these are therefore listed as Unknown. A corresponding "Unknown" designation is also listed in the Specific Location column.

3. Zone Color—four colors were identified for specific areas on the floor 5: green/Daschle, orange/contaminated yellow/unlikely, red/proximate.

4. Specific Location—the surface that was sampled within an area is referred to as the specific location (Wall 1, Wall 2, etc.). The original identifying numbers from the SampleID were retained in this column.

5. Key Description—this is a cross reference to the *Spore Strip Location* file using the Specific Location column and it provides more descriptive definitions (for example, Wall – back, Drawer – open desk, etc.). If there was no corresponding key description in the *Spore Strip Location* file, it was listed as Unknown.

6. General Location—in order to obtain an overall view of all the walls, floors, etc., this column was created to group specific locations.

7. On Location List—a few items were not listed in the *Spore Strip Location* file and these were identified with a "no" in this column.

8. Bacteria/Concentration—this data was pulled directly from the SampleID and in a few instances (for example, DT3, C, C1, C2, C3), the code for the organism and concentration appears to have typographical errors in the original file. There was no attempt to correct for this. C, C1, C2, and C3 all had negative growth.

9. Bacteria Group—this column summarized the Bacteria/Concentration column so the bacteria could be consolidated, regardless of concentration (for example, all the *B. subtilis* data could be filtered or have a PivotChart created). This column also clearly differentiates between spore strips and Steri-charts. If Unknown appears, this was one of the instances (for example, DT3, C, C1, C2, C3) in which the organism could not be identified.

Worksheets 2-5. PivotChart-Bact by Key Descr, PivotTable-Bact by Key Descr, PivotChart-Bact by Gen Loc, PivotTable-Bact by Gen Loc. To visualize the data in the *Complete Data* worksheet, PivotChart and PivotTable reports were created to aid in summarizing and obtaining alternate views of the data. To illustrate the different ways PivotTables and PivotCharts can be used, two versions of each type were created: *Bacteria by Key Description* (Worksheets 2&3) and *Bacteria by General Location* (Worksheets 4&5).

PivotTable and PivotChart reports are interactive tables and charts that summarize large amounts of data and allow a user to display different details of the data quickly and easily. PivotChart reports are graphical views of data and PivotTable reports are tabular views of the data. A PivotChart is always associated with a PivotTable report and these two reports are based on the same source data. The PivotTable toolbar allows the user to change the arrangement of the data that appears in the report by dragging different fields directly into and out of the report. Each field corresponds to a column in the source data, in this case, Worksheet 1, *Complete Data*. Different data within the field can be selected by using the field buttons, and identified by the down arrows next to the field name, once the fields are on the report layout.

Example. Worksheet 2&3, Bacteria by Key Description. These reports illustrate and summarize data by looking at the location of the spore strips and Steri-charts at as specific a location as possible, the Key Description. Area is the page field and Cubicle 12/60 is the selected item in the page field on the report. Note that the floating Pivot Table toolbar is only used to drag fields to and from the report and is not used to change the selections within a field. The graph and chart summarize the bacteria group information only for that area – the area can be changed by simply selecting a different location by clicking the down arrow on the area field button on the report. In the *Bacteria by Key Description* report, the Bacteria Groups *B. subtilis*, *B. stearothermophilus* 10^5, and *B. stearothermophilus* 10^6 have been selected (although more or less could be selected by simply selecting and deselecting boxes using the Bacteria Group field button) as found by the Key Description field (all fields currently selected). The actual data that is being viewed is the positive (red) and negative (green) growth that occurred on Day 7. What is quickly clear from this graphic is that most of the bacteria survived ClO_2 treatment and were growing after the 7th day of culture. Various areas, key descriptions, bacteria groupings, and types of growth on Day 7 can all be changed without changing the layout of the report format by using the appropriate field buttons.

Worksheet 6. ***Lookup Tables.*** These were used in the formulas when creating the new columns in the *Complete Data* worksheet.

No key findings were observed from this extensive analysis of the data. Therefore, this information is presented as *Appendix 6* found in the CD version of the report. The numerous graphs and figures can be viewed and manipulated by the reader in this format.

Because the chlorine dioxide had not been employed as a disinfectant at this scale ever before, it was important to determine if the chlorine dioxide gas treatment was more efficient in some special configurations verses others. The data in the Washington, DC, trailer location indicated effective killing of dried spores, but the size of that trailer was more than an order of magnitude less that the office area targeted for chlorine dioxide gas remediation. Therefore, it was important to determine if vertical or horizontal surfaces were more effectively remediated by the gas.

Because the spores were released as a fine dry powder, they were identified on both horizontal (floors, desk tops, mailboxes) and vertical surfaces (walls and computer screens). The extent of contamination was greatest on the horizontal surfaces, partially due to the effect of gravity pulling the fine powder to the earth. Knowing that the chlorine dioxide gas is slightly heavier than air, it seemed logical that the kill rate on horizontal surfaces may exceed that of the vertical surfaces.

Close examination of the office data demonstrated no clearly observable trends regarding a correlation between effectiveness of kill and the spatial placement of the spore strips. While this was not totally unexpected, some anomalies were observed within the various data sets that led to further analysis.

A location-by-location analysis of the spore strip data revealed that a few specific locations appeared to have the majority of strips that were positive for growth. Indeed, area 12 and the stairs had a striking number of positive cultures. In addition, a significant number of the negative controls from these areas demonstrated positive growth. The negative control strips

contain no inoculated bacteria and are used as an indication of proper handling and processing of the spore strips. The vast majority of the negative control strips indeed yielded no growth during the 7-day incubation as anticipated. However, location 12 and the stairs had 13 of 16 and 10 of 18 negative controls generating growth. Thus, it appears that the positive spore strips in these two locations were probably due to adventitious contamination by bacteria rather than to the presence of surviving surrogate *Bacillus* spores.

A preliminary analysis of the spore culture results by individual rooms is presented in *Appendix 11*. This table presents the data as log spore kill as a function of specific areas within the fifth and sixth floors. The killing efficiency on the sixth floor was significantly (one to two logs) greater than that observed on the fifth floor. Analysis of spore strips from the fifth floor indicates a lack of effective sterilization with the majority having a four logs or lower killing efficiency.

Unfortunately, there was no culture verification to identify the organism(s) growing in the nutrient broth from any of these locations. Therefore, these results are uninformative with respect to ascertaining the effectiveness of the fumigation in these areas. However, when the SwRI® laboratory cultured positive cultures from later data generated at the Beltsville trailer location, the majority of the characterization cultures indicated there was an organism other than the indicator species present. This suggests a contamination artifact rather than ineffective killing by the chlorine dioxide gas.

While it is not possible to definitively determine from the available data where contamination was introduced along the chain of custody, there are some possible explanations as to the source of the contamination. One would be that if the growth from the negative control strips was the result of a random contamination, such as an artifact of poor handling of the samples prior to arrival of the spores to the analytical laboratory, the positive cultures from the negative controls would not be as localized as they appear to be. The localization is consistent with either onsite contamination, such as that introduced by walking on the spore strips and disrupting their integrity post fumigation, or packing/unpacking of specific bundles on the way to or at the analytical laboratory. The site was very crowded with tubing, cords, wiring, etc., making traversing the area in Level A suits a daunting task.

Another possible explanation for the data anomalies may have been obtained from the *B. stearothermophilus* spore strips. These organisms can provide key information regarding exterior contamination because of their requirement to be cultured at 60°C. Most of the contaminating organisms cannot grow under these relatively extreme conditions. Therefore, if a spore strip contained killed *B. stearothermophilus* spores, no growth would occur even if it was contaminated because the contaminating organisms would not grow at the elevated temperature. Standard culture conditions are 37°C. Unfortunately, the SwRI® analysis laboratory incubated all cultures at 37°C for the first 48 hours before understanding the elevated temperature requirements for *B. stearothermophilus*. All cultures containing the *B. stearothermophilus* spore strips in area 12 were positive by this incubation time. Therefore these data did not help the analysis.

The USEPA conducted clearance sampling of the contaminated area following fumigation. The results of this analysis demonstrated that in the regions of highest contamination there were still viable spores. The USEPA conducted spot remediation of these areas with chlorine dioxide in

solution. Subsequent clearance sampling demonstrated no viable spores. An advantage of the chlorine dioxide is that while it kills the spores, it does not destroy the nucleic acid within the spore. Therefore the samples could be demonstrated to contain genetic information from *B. anthracis*, but the material was from dead spores. This demonstrates that the spores were actually killed and not just removed during remediation activities.

Ultimately, it was the use of clearance sampling to demonstrate the absence of viable organisms from any location within the building, surfaces or air, that allowed for the determination that the overall remediation was successful and that the building could be safely reoccupied. This approach provided the Review Working Group with sound, scientific data with which to recommend that the Incident Commander turn control of the building over to the AOC to proceed to reoccupy (*Appendix 12*). The HSOB was reopened on January 22, 2002.

3.6 HSOB HVAC Fumigation Activities and Results

The air handling systems supplying the Daschle suite in the HSOB were shut down within less than an hour after detecting anthrax contamination. Subsequent sampling and analysis indicated contamination in air return vents from the affected suite as well as within the return side of the associated AHUs. While the levels of contamination were not judged to be high, the potential for additional circulation of contamination warranted significant caution. No quantified levels of contamination were provided, and multiple rounds of sampling and analysis were negative in regions first identified as contaminated. The findings that secondary sampling was negative were not unusual. The consensus from these results in the office regions of the building was that the initial sampling actually removed all of the measurable contamination.

A fumigation was initially performed on the HVAC system for the most highly contaminated area between December 14, 2001, and December 17, 2001. The SOP for operations is found in *Appendix 13*. This first attempt to fumigate the HVAC was unsuccessful due to a mechanical blockage that impeded the flow of ClO_2 and less than optimal temperature and humidity levels. A second fumigation of the HVAC system was carried out between December 27, 2001, and December 31, 2001. During both fumigations, negative air machines pulled 150 cubic feet of air per minute through the HVAC system. Residual ClO_2 in the exhaust from the machines was scrubbed and vented to the atrium of the HSOB. The same process was used to remove the ClO_2 gas from the HVAC system upon completion of each fumigation. Air monitoring and operational sampling was used to monitor gas concentration, temperature, and humidity throughout the process.

The placement of spores within the HVAC system was complicated by the limited access to the actual vents. Figure 10 demonstrates that strips were clipped to metal rods that were placed horizontally at every other floor to verify the remediation process.

During the entire fumigation process the temperature, relative humidity and chlorine dioxide levels were monitored in either real-time or near real-time. Given the vertical nature of a typical HVAC system for a tall building, the maintenance of constant environmental conditions was an issue. The relative humidity in one region was below 70 percent for a majority of the fumigation and this area was spot cleaned with liquid decontamination solution. As anticipated, the spore strips in this location demonstrated less than a 10^6 killing efficiency.

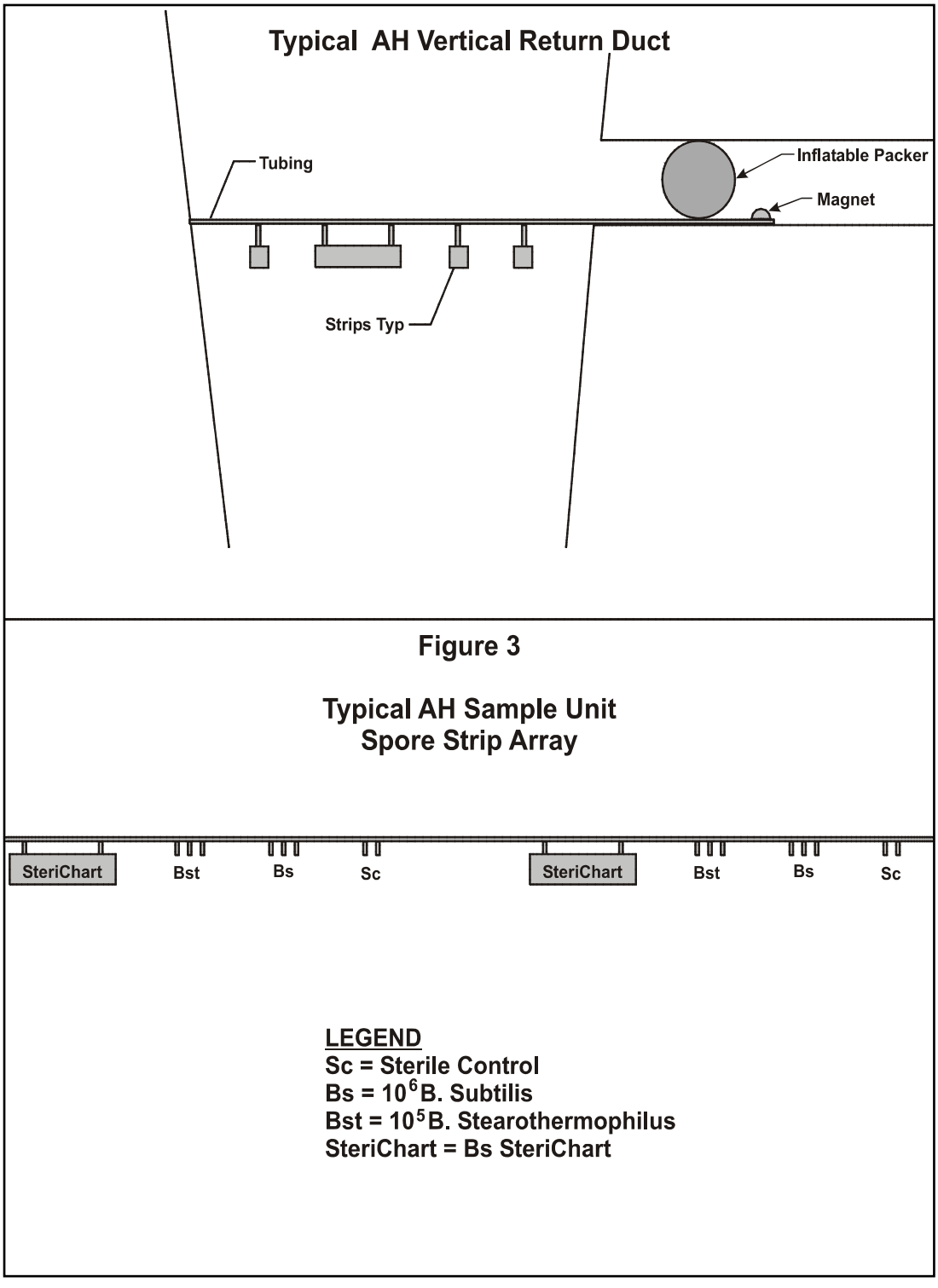


Figure 10. Diagram of Spore Strip Placement in HVAC System.

Two *Bacillus* species, *B. subtilis* and *B. stearothermophilus*, were used to determine the effectiveness of the ClO_2 gas fumigation of the HVAC system. A total of 440 spore strips were positioned at 11 locations in the HVAC system. Of those, 395 were recovered after the second fumigation and an additional 33 were recovered later. The spore strips were handled in a manner similar to the strips from the initial remediation in the contaminated suite. Most of the strips were processed and analyzed at SwRI®, but some were sent to Dugway Proving Ground to decrease the burden on SwRI®. Spore strip analysis and verification sampling both indicated that the fumigation had demonstrated the inability to find any culturable organisms from surfaces or air. Actual results from the spore strip culturing are presented in *Appendix 14*.

In a USEPA sponsored Final Report of the HVAC remediation efforts (*Appendix 15*), authors Steven Hirsh, USEPA Region III; David Skodack, Tetra Tech EM, Inc, and John Young Mason, Sabre Oxidation Technologies Inc., dated February 2002 concluded: "A second HVAC fumigation occurred between December 28th and December 31st 2001. Environmental samples and spore strip analysis indicated that the fumigation achieved an effective remediation of the HVAC system. The fumigation achieved Food and Drug Administration (FDA) standards for sterilizing levels of sporicidal effects (10^6 *Bacillus subtilis* (Bs) kill, 10^5 *Bacillus stearothermophilus* (Bst) kill), (Leighton 2002)." Analysis of the spore strip results indicated relatively homogenous effects. Since all strips were suspended in the air handling regions, there was no attempt to determine if the middle or the sides of the chambers had better killing efficiencies. All regions achieved sufficient kill levels.

"Post-fumigation environmental and air sampling suggested that the ClO_2 fumigation was successful in remediating the spore burden within the HVAC system (Leighton 2002)."

The remediation of the air handling systems was not without difficulty. The first attempt was aborted when the required relative humidity levels could not be achieved. It is important to note that the relative, not absolute humidity, is the important value. As the air temperature increases it takes more water in a given volume of air to maintain a constant relative humidity. One of the regions of the air handling system had lower levels of humidity than optimal. Prior to obtaining spore strip results, the USEPA team remediated all surfaces in that region with a chlorine dioxide containing liquid. As anticipated, the spore strip killing efficiency was low but subsequent clearance sampling indicated the absence of *B. anthracis* spores.

3.7 Beltsville Trailer Tests

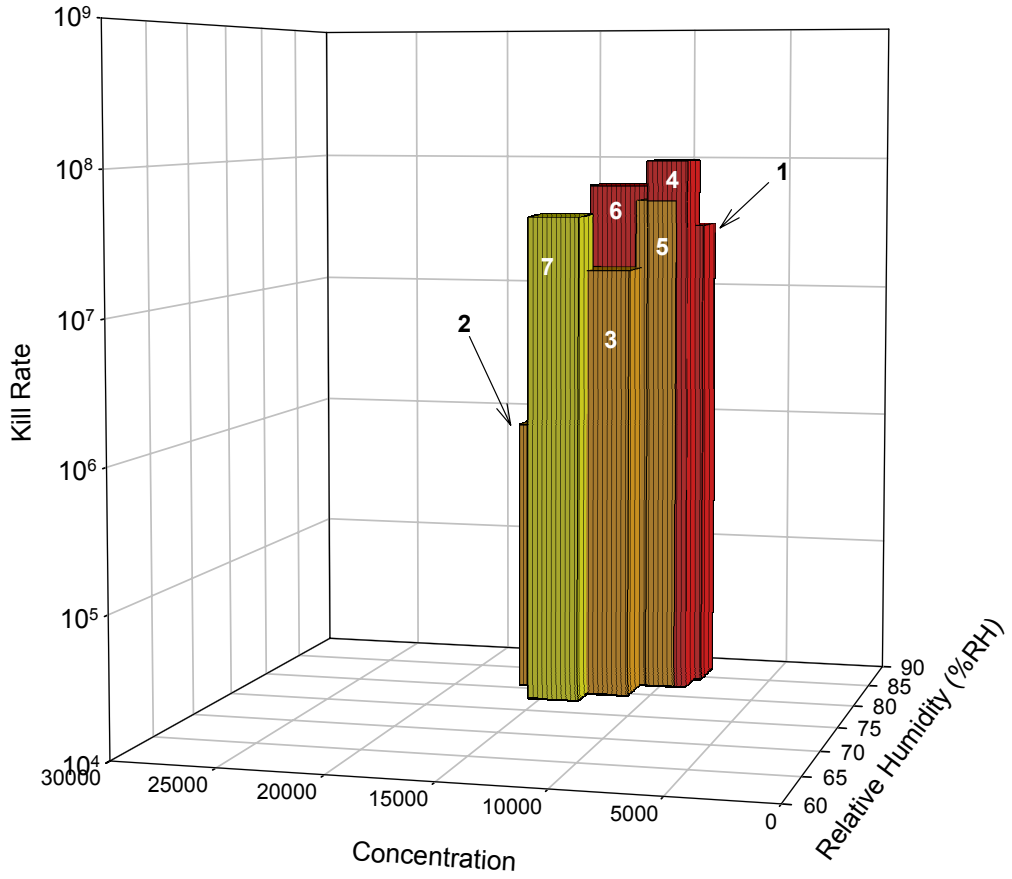
After fumigation of the HSOB, USEPA conducted a series of seven fumigations in a trailer at the U.S. Department of Agriculture (USDA) Agricultural Research Center in Beltsville, Maryland, to decontaminate U.S. mail and private carrier packages transferred from the P Street Warehouse as well as artifacts, critical items, and other items that were not successfully treated with ethylene oxide sterilization. The first six runs runs were conducted between March 22 and March 28, 2002. The seventh run was conducted on April 10 and 11, 2002. The target exposure concentration was 1,000 ppm for 9 hours in runs one through six. In the seventh run, the target ClO_2 concentration was 450 ppm for a longer exposure time of 20 hours. Uncontaminated packaging materials were included in the last run to determine the penetration efficiency and other effects of ClO_2 on various materials. The target temperature for all runs was approximately 80°F, with a target relative humidity of 80 to 85 percent. Actual measurements were taken for all three parameters at frequent intervals during each run.

Spore strips were used to determine the effectiveness of the fumigations. As with other fumigations, *B. subtilis* and *B. stearothermophilus* were used. A combination of spore strips and Steri-charts were placed in 30 designated sampling locations in the treatment trailer during each run. The effectiveness of each fumigation was assessed using a total of 255 spore strips. Each array consisted of a negative control strip, three *B. subtilis* spore strips, and one *B. stearothermophilus* spore strip. Steri-charts were included at half of the sampling locations, along with an additional negative control strip. Spore strips were in a Tyvek sleeve and all samples were handled with powder-free, sterile, nitrile gloves and alcohol-sterilized tweezers to prevent cross-contamination. Each spore strip included a location code and/or a unique identification number. Positive controls from each Steri-chart were used to assess viability of the spores on the test strips. Thus, they were removed and placed in a pre-labeled key envelope prior to positioning of the charts and were never exposed to ClO_2 gas.

Sporicidal efficiency was calculated for each individual run for each of the two indicator organisms. The use of the Steri-chart strips allowed for quantification of efficiencies of kill up to 10^8 for *B. subtilis* and 10^7 for *B. stearothermophilus*. Fractional exponents were calculated based on the raw data. In some cases there was positive growth at 10^6 with both higher concentrations being growth negative. This could be the result of clumping on the spore strip, or handling and/or laboratory error. If the sample was subsequently analyzed by culture and the resultant organisms were not the indicator species, the culture data were considered growth negative. There were no cases in which growth at lower concentrations was the target organism.

Two Beltsville 3-D bar graphs were generated illustrating five factors: relative humidity, temperature, concentration of chlorine dioxide integrated over time (CT), log kill rate, and organism type (Figure 12, *B. subtilis,* and Figure 13, *B. stearothermophilus*). The graphs were generated from data in the table inserts of the figures. The graphs present kill rate, concentration of chlorine dioxide integrated over time (CT), and relative humidity (%RH) on three axes. The

Figure 11. Chlorine Dioxide Generation System and Interior Operations. *These two photos illustrate the chlorine dioxide generation system and the interior setup at the Beltsville remediation facility.*

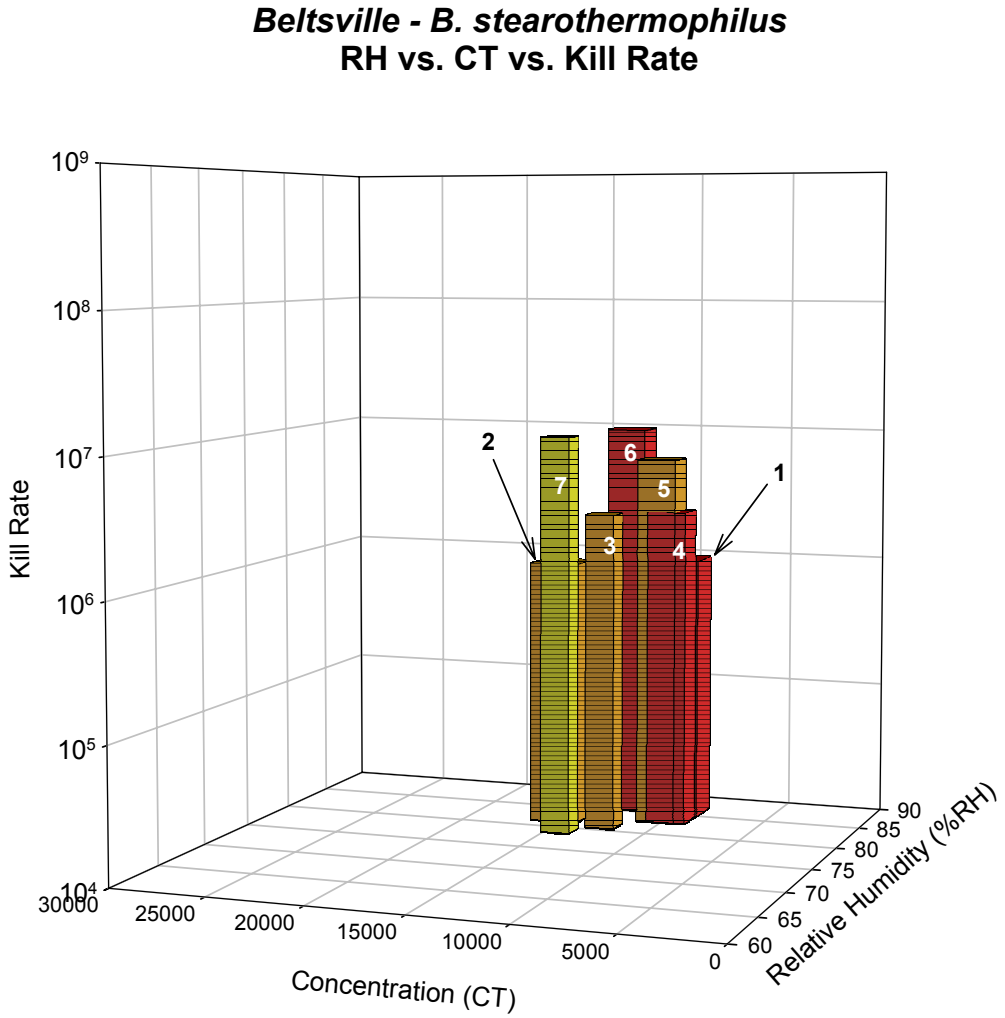


Beltsville—*B. subtilis*

Test Run	%RH	Temp (°F)	CT	Kill Rate	Run Duration (hours)	CT/Hour
1	86.0	85.7	9,920	$10^{7.5}$	9.7	1022
2	82.4	84.6	15,345	10^{6}	12.0	1278
3	81.1	84.4	12,578	$10^{7.2}$	9.0	1397
4	83.8	85.7	9,772	10^{8}	7.7	1269
5	83.8	84.1	10,325	$10^{7.7}$	8.0	1290
6	86.3	87.4	12,722	$10^{7.8}$	9.0	1413
7	79.3	80.2	14,374	$10^{7.6}$	21.0	684

Temp Range (°F)	Color Key
<75.1	
75.1-77.5	
77.6-80.0	
80.1-82.5	
82.6-85.0	
>85.0	

Figure 12. Beltsville—B. subtilis. *B. subtilis killing activity during runs 1 through 7 at Beltsville.*



Beltsville—*B. stearothermophilus*

Test Run	%RH	Temp (°F)	CT	Kill Rate	Run Duration (hours)	CT/Hour
1	86.0	85.7	9,920	10^6	9.7	1022
2	82.4	84.6	15,345	10^6	12.0	1278
3	81.1	84.4	12,578	$10^{6.4}$	9.0	1397
4	83.8	85.7	9,772	$10^{6.4}$	7.7	1269
5	83.8	84.1	10,325	$10^{6.8}$	8.0	1290
6	86.3	87.4	12,722	10^7	9.0	1413
7	79.3	80.2	14,374	10^7	21.0	684

Temp Range (°F)	Color Key
<75.1	
75.1-77.5	
77.6-80.0	
80.1-82.5	
82.6-85.0	
>85.0	

Figure 13. Beltsville—B. stearothermophilus. *B. stearothermophilus killing activity during runs 1 through 7 at Beltsville.*

fumigation temperatures are color coded in incremental ranges from less than 75.1°F (blue) to greater than 85°F (red). The organisms are identified with different patterns on the bars of the graph. *B. subtilis* has vertical lines and *B. stearothermophilus* has horizontal lines. The same scaling was used on the axes of the two graphs to allow for easier comparison.

Temperature and Relative Humidity Data. The files *32302 Run 1*, *32402 Run 2*, *32502 Run 3*, *32602 Run 4*, *32702 Run 5*, *32802 Run 6*, *41002 Run 7* were used to determine temperature values listed in Figures 12 and 13. Temperature and relative humidity values were calculated by taking the average of the readings for each data run from two machines, Model No. 8762, for the "Black" version (Serial No.: 01120527) and the "Grey" version (Serial No. 01120217). The temperature and humidity values used ranged from the time of initial treatment of ClO_2 to the final reading. After the average was obtained for each machine, an average of the two values was taken for the final temperature and relative humidity value listed for each test run. Actual recordings are presented in *Appendix 19*.

Concentration Data. The file "Beltsville CT Data Final" was used to determine concentration values listed in Table 1 and Table 2. Concentration values were calculated by taking the average of the final concentration readings of the four sample locations for each test run.

Summarized spore strip culture data in Excel format are contained in *Appendix 16*. Some of the positive culture tubes were selected for subsequent analysis to determine if the growth was resultant from the indicator organism. The laboratory tested a subset of the cultures that were growth positive to determine if the organism responsible for the growth was indeed the indicator species on the spore strip. The procedures followed are found in *Appendix 17*. These results are presented in *Appendix 18*. As one can see, the predominant bacteria responsible for the positive results are not the same organism as was present in the spore strip. These results indicate contamination of the spore strips prior to inoculation of the growth media. These results raise serious questions about the entire handling and processing of the spore strips. In order to address this issue, the laboratory conducted growth assays from over 100 negative cultures with only one false positive. This suggests, but does not prove, that the strips were contaminated prior to the point of culture inoculation.

B. subtilis var. *niger* was originally selected as a surrogate organism at what was then Camp Detrick because of its unique property of producing an orange color on agar growth plates (see lower figure on document cover). This makes the indication of this particular strain relatively easy in a subsequent culture analysis.

Run #1

The first run resulted in a chlorine dioxide CT value of 9,920 over a 9.7-hour period with an average concentration of 1,022 ppm per hour. The average temperature was 85.7°F with an average RH of 86 percent. All of these values were within the target range. The kill rate for *B. subtilis* was $10^{7.5}$ and the kill rate for *B. stearothermophilus* was 10^{6}.

Run #2

The second run resulted in a relatively high chlorine dioxide CT value of 15,345 over a 12-hour period with an average concentration of 1,278 ppm per hour. The average temperature was

82.4°F with an average RH of 85 percent. All of these values were within the target range. The kill rate for *B. subtilis* was 10^6 and the kill rate for *B. stearothermophilus* was also 10^6.

The efficiency of kill of *B. stearothermophilus* for the second run at the Beltsville location is analogous to the results from run 1. The *B. subtilis* killing efficiency was notably low in the second run. The temperature and humidity were within the target range. The CT value was actually 50 percent higher than the target level. The elevated chlorine dioxide concentration would lead one to predict a better killing efficiency based on previous data. In fact, the killing efficiency was 10^6, but this was still below all other runs. Upon careful evaluation of the spore strip analysis, a potential explanation is evident. On this run the spore strips appear to be grouped by type and number of organisms rather than by location. For all other runs the spore strips were analyzed in groupings by location rather than by type. Stated in another way, spore strips containing the same amount of a particular bacterial spore were processed together. As a result of this organization, the spore strips containing 10^7 and 10^8 spores were processed at the end of the run. All of the last 21 samples prior to the positive controls demonstrated growth, including a negative control that was also positive.

It is possible that an artifact in the strip processing created this large cluster of positive cultures. With the available data, it is impossible to determine the source of the contamination. None of these positive cultures were analyzed to determine if the bacteria were the same bacteria as present on the original spore strip. Therefore, it is likely that the killing efficiency for Run #2 is actually better than depicted in Figure 12 and Figure 13.

Run #3

The third run resulted in a chlorine dioxide CT value of 12,578 over a 9-hour period with an average concentration of 1,397 ppm per hour. The average temperature was 84.4°F with an average RH of 81 percent. All of these values were within the target range. The kill rate for *B. subtilis* was $10^{7.2}$ and the kill rate for *B. stearothermophilus* was $10^{6.4}$.

Overall, runs 1 through 3 had the lowest killing efficiencies for both target organisms.

Run #4

The fourth run resulted in a chlorine dioxide CT value of 9,722 over a 7.7-hour period with an average concentration of 1,269 ppm per hour. The average temperature was 83.8°F with an average RH of 86 percent. All of these values were within the target range. The kill rate for *B. subtilis* was 10^8 and the kill rate for *B. stearothermophilus* was $10^{6.4}$.

While this run had the lowest CT value, it had the most effective kill rate for *B. subtilis*. The killing efficiency for *B. stearothermophilus* was also high, but not the highest in the series. The high temperature and relative humidity may have been responsible for the enhanced performance.

Run #5

The fifth run resulted in a chlorine dioxide CT value of 10,325 over an 8-hour period with an average concentration of 1,290 ppm per hour. The average temperature was 84.1°F with an

average RH of 84 percent. All of these values were within the target range. The kill rate for *B. subtilis* was $10^{7.7}$ and the kill rate for *B. stearothermophilus* was $10^{6.8}$.

The operating conditions for runs 4 and 5 are essentially identical and the killing efficiency in the two runs is not notably different. Thus, the results may illustrate the range of values that can be expected under nearly identical conditions.

Run #6

The sixth run resulted in a chlorine dioxide CT value of 12,722 over a 9-hour period with an average concentration of 1,413 ppm per hour. The average temperature was 86.3°F with an average RH of 87 percent. All of these values were within the target range. The kill rate for *B. subtilis* was $10^{7.8}$ and the kill rate for *B. stearothermophilus* was 10^{7}.

This run demonstrates among the highest killing effect for both indicator organisms. It may be important to note that this run has the highest temperature and relative humidity of all the runs.

Run #7

The seventh and final run resulted in a chlorine dioxide CT value of 14,374 over a 21-hour period with an average concentration of 684 ppm per hour. The average temperature was 80.2°F with an average RH of 79.3 percent. All of these values were within the target range. The kill rate for *B. subtilis* was $10^{7.6}$ and the kill rate for *B. stearothermophilus* was 10^{7}.

This final run was unique in numerous aspects. While it had the second highest overall CT value, the actual chlorine dioxide concentration within the enclosure was almost one-half of the concentrations of the previous six runs. In addition, this run had the lowest temperature and relative humidity values. Even with these lower temperatures and absolute chlorine dioxide concentrations, the killing efficiency was equivalent to the most effective kills measured in other runs. Although this is a single experiment, the data are clearly consistent with the concept that it is the overall chlorine dioxide exposure levels, not the absolute levels that provide the most significant spore killing capability.

Summary of Beltsville Operations

The operational parameters of chlorine dioxide concentration, time of exposure, temperature, and relative humidity chosen for the fumigation runs at Beltsville were all successful in achieving a minimum of a 10^{6} kill of the indicator organisms in a reproducible fashion. Although data from Brentwood P&DC trailer tests and the HSOB fumigation indicated the importance of humidity and temperature, there was not an observable temperature or humidity enhancement in the operational ranges tested at Beltsville. In fact, one of the best kill rates was obtained at the lowest temperature and relative humidity. This final run also employed a significantly lower absolute concentration of chlorine dioxide, but with an increased exposure time resulting in an increased final CT value. These conditions also resulted in an excellent killing efficiency.

3.8 Supporting Documentation from Dugway Proving Ground

In order to further document the effect of gaseous chlorine dioxide on *B. anthracis* spores, the USEPA in Denver, Colorado, contracted the West Desert Test Facility at Dugway Proving Ground to test the effects of the gas on a variety of dried *Bacillus* spores. A copy of "The Abbreviated Test Report for the Laboratory Validation of Chlorine Dioxide Decontamination" is included in this report as *Appendix 1*. The findings support and strengthen the conclusions drawn from the test data at the Brentwood P&DC and Beltsville test facilities.

The data discussed and illustrated in this section were excerpted from "Abbreviated Test Report for the Validation of Chlorine Dioxide Decontamination," test project No. 8-CO-210-000-084, WDTC document No. WDTC-TR-02-059, August 2002.[1]

Spores from three strains of *B. anthracis* (BAA – *B. anthracis* var. *ames,* BAV – *B. anthracis* var. *vollum,* BAS – *B. anthracis* var. *sterne*) and three *Bacillus* simulants (BGN – *B. subtilis* var. *niger,* BT – *B. thuringiensis,* BST – *B. stearothermophilus*) were dried on either glass slides or porous filter paper and exposed to chlorine dioxide gas for 1, 2, 4, 6, 8, and 12 hours at different relative humidities (30 to 92 percent). One key observation from the analysis of these data is that the Dugway team did not control for temperature. Discussions with the Dugway team indicated that they operated at ambient temperatures (70 to 75°F).

Spore preparations were cultured and a liquid slurry was applied to either porous filter paper or glass slides and dried. Triplicate slides or filter paper were removed from the sterilization chamber at specific intervals and cultured to determine presence of viable spores. Experiments were conducted at various chlorine dioxide concentrations as well as various relative humidity values.

The Dugway team noted that for all organisms tested, the relative humidity was more important than the chlorine dioxide level for killing of all three strains of *B. anthracis* spores. This was reported for concentrations ranging from 125 to 1050 ppm. The authors recommend a relative humidity of greater than 70 percent for effective spore killing activity.

The authors stated that the spores dried on the glass slides were more resistant to the remediation. This is very understandable since the spores are known to clump when wet and most likely provided a level of insulation for spores farther away from the air interface. The spore preparations on the filter papers were not subjected to analysis because the majority demonstrated a 10^6 kill after only one hour under most conditions. It is important to note that the filter paper preparations were most like the conditions at the HSOB. The spore preparation in the envelope was reported to be very dry. It is possible that some spore clumping occurred in regions where sampling was conducted, but this was most likely minimal.

Another interesting finding from the Dugway report is that their data using the spores containing *B. subtilis* var. *niger* were variable to the point to preclude including the results for analysis. This is the same strain used by the USEPA to validate the chlorine dioxide remediation using spore strips. While the data for *B. subtilis* were reported to be variable in the Dugway report, the

[1] The USEPA in Denver, Colorado, funded this work and distribution is authorized to U.S. Government agencies only. Other requests for the entire document should be referred to USEPA, 999 18th Street Suite 300, 8EPR-ER, Denver, CO 80202.

experimental conditions that were most closely aligned with the conditions at the HSOB fumigations demonstrated effective kill against actual and surrogate spores. These results suggest that the *B. subtilis* may not have been the most appropriate strain as an indicator of *B. anthracis* spore killing. It should be pointed out, however, that two other strains of *Bacillus* spores were also used for monitoring of the HSOB operations – *B. stearothermophilus* and *B. thuringiensis*. In addition, surface and air clearance sampling that demonstrated no growth on any samples confirmed the overall effectiveness of the HSOB fumigations.

The complete Dugway report is included as *Appendix 1*. Six of the data sets that are most illustrative of the effects of humidity and total chlorine dioxide exposure have been included.

Figure 14 presents Trial 11, which indicates that at 30 percent RH a modest level of ClO_2 has no measurable effect on viability of any spore type tested. Figure 15 presents Trial 9, also at 30 percent RH, which demonstrates that at higher levels of ClO_2, 1,050 ppm, there is significant loss of viability as a function of time, but only for the BGN spores. These data support the observation that the BGN is the most susceptible spore tested to ClO_2.

Figure 16 presents Trial 5, which utilized an intermediate concentration of ClO_2, 613 ppm, at 60 percent RH. At this higher RH value and a moderate level of ClO_2, only BGN was susceptible to the gas. These results further support the observation that BGN is the most susceptible spore tested to ClO_2 effects. These data strongly support the requirement for a RH above 60 percent for effective sporicidal activity using ClO_2.

Figure 17 presents Trial 1, which utilized an intermediate concentration of ClO_2, 650 ppm, at a RH of 75 percent. Under these conditions the BGN was again most susceptible. No viable

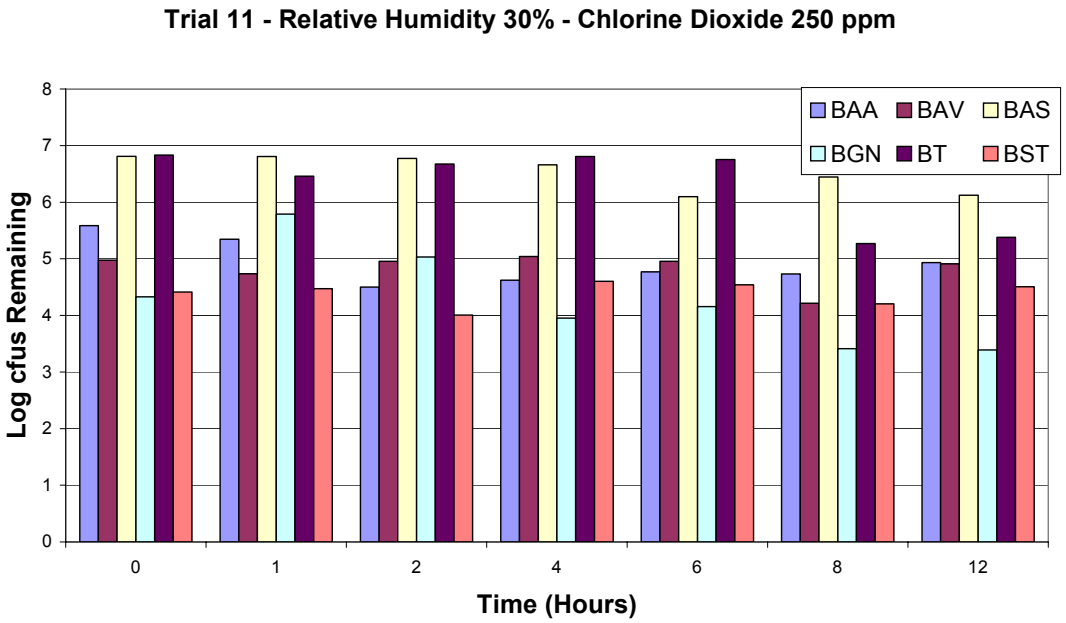


Figure 14. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 11; Laboratory Validation of Chlorina Dioxide Decontamination.

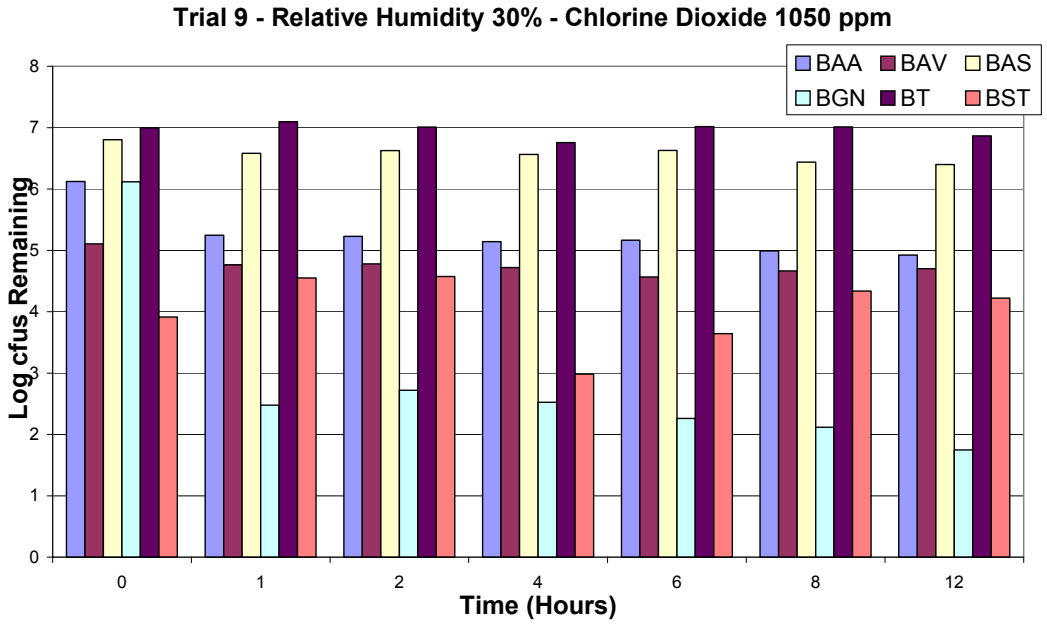


Figure 15. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 9; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames,* BAV – *Bacillus anthracis* var. *vollum,* BAS – *Bacillus anthracis* var. *sterne,* BGN – *Bacillus subtilis* var. *niger,* BT – *Bacillus thuringiensis,* BST – *Bacillus stearothermophilus.*

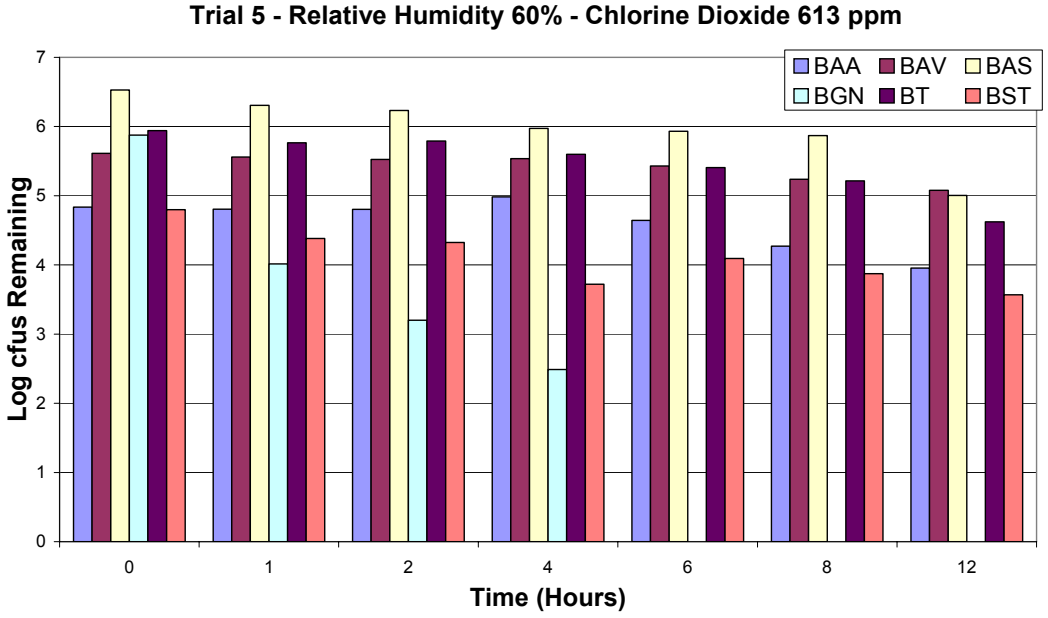


Figure 16. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 5; Laboratory Validation of Chlorine Dioxide Decontamination.

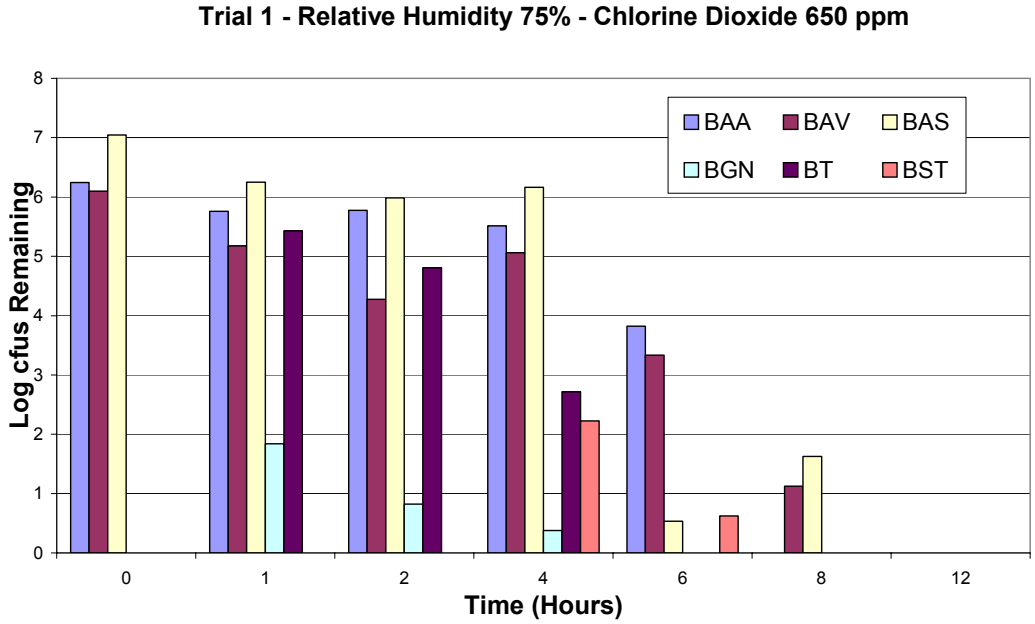


Figure 17. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 1; Laboratory Validation of Chlorine Dioxide Decontamination.

spores were observed of any type after 12-hour incubation. These conditions correlate to a CT value of 7,500. These conditions are very close to the target conditions for remediation of the HSOB by the USEPA. The first four data sets clearly demonstrate that relative humidity, time, and ClO_2 concentration are very important for effective remediation with ClO_2.

Figure 18 clearly identifies relative humidity as the most critical parameter for the ability of ClO_2 to kill *Bacillus* spores. At a relatively low concentration of ClO_2, 250 ppm, and a 90 percent RH there were essentially no viable spores of any type remaining after two hours of incubation. These conditions were more than twice as effective as the data with a RH of 75 percent and a greater than two-fold increase in ClO_2 concentration.

The experiments conducted at Dugway Proving Ground clearly demonstrate the importance of relative humidity, time, and ClO_2 concentration for the killing of dried *Bacillus* spores. In addition, the data clearly support the use of ClO_2 gas for remediation of material contaminated with *Bacillus* spores.

The data presented were for spores dried on glass slides. The scientists also conducted parallel experiments using the same spore slurry dried on filter paper. As one can easily visualize, the spores on the filter paper had a greater overall surface area as they adhered to the 3-D fiber matrix of the paper. Thus, these spores were readily exposed to the ClO_2 gas from all sides. In contrast, the spores on the glass slide were only accessible to ClO_2 gas from one side and may have been susceptible to clumping. The data obtained from the filter paper indicated almost 100 percent killing of all spore types by one hour under almost all experimental conditions, whereas the data for the glass slide indicated much longer kill times. This has significant bearing on the use of ClO_2 gas for building remediation of *Bacillus* spore contamination.

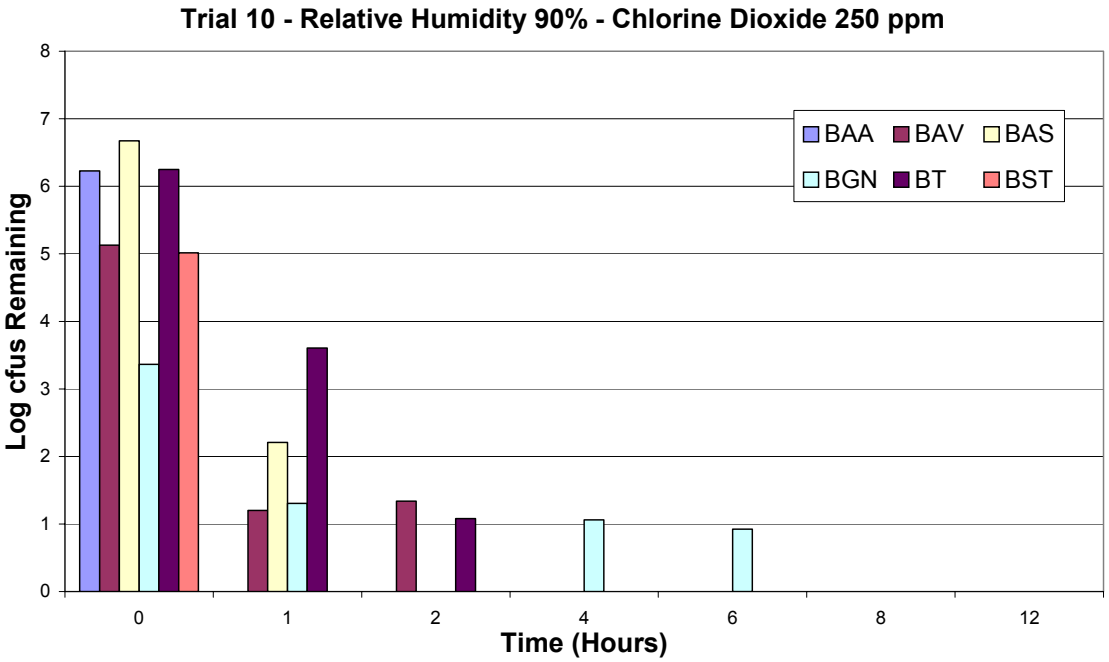


Figure 18. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 10; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames,* BAV – *Bacillus anthracis* var. *vollum,* BAS – *Bacillus anthracis* var. *sterne,* BGN – *Bacillus subtilis* var. *niger,* BT – *Bacillus thuringiensis,* BST – *Bacillus stearothermophilus.*

The attack with *B. anthracis* spores at the HSOB involved dried, weaponized spores. As these spores were released into the atmosphere they slowly settled as a result of gravity. However, these spores were readily reaerosolized with the slightest disturbance. Reports were published in the Journal of the American Medical Association "Secondary Aerosolization of Viable *Bacillus anthracis* Spores in a Contaminated US Senate Office**"** JAMA. 2002; 288:2853-2858. Also, they were not deposited on surfaces in a liquid state that then dried, as was done with the spores deposited on glass slides in the Dugway tests. Therefore, the dispersal of dried spores in a building may more closely represent the spores dried on filter paper rather than a glass slide.

The effort at Dugway Proving Ground, funded by the USEPA, was not completed before the USEPA had to establish conditions to fumigate the HSOB. Multiple tests were run at the Brentwood P&DC trailer test facility in order to determine the environmental conditions necessary for effective *Bacillus* spore killing by ClO_2. These experiments are documented in "Chlorine Dioxide (ClO_2) Gas Phase Demonstration Project," and are discussed in this document.

While the conditions evaluated at the Dugway test site did not exactly mimic the conditions employed at the HSOB, the Dugway laboratory test results demonstrate effective killing of live *B. anthracis* and surrogate spores at 75 percent RH and 75°F at 650 ppm chlorine dioxide after 12 hours exposure. Under these conditions all of the spores dried on a cover slip were killed.

The report also stated that the spores dried on filter paper were so susceptible to killing by chlorine dioxide that most of the data were omitted from the report.

The reported conclusions from the USEPA commissioned laboratory analysis of chlorine dioxide remediation abilities is very supportive of the results obtained in the trailer test facilities. These results indicate that the temperature and relative humidity, along with the chlorine dioxide levels achieved in the remediation of the HSOB were sufficient to achieve at least a 10^6 kill of any *B. anthracis* spores present. Accordingly, the Dugway test data provide further evidence that the conditions utilized for fumigation operations at the HSOB were effective against *B. anthracis* spores.

4. OVERALL CONCLUSIONS

The events surrounding the contamination and cleanup of the greater Washington, DC, area from *B. anthracis* spores were unprecedented. As such, they presented unique challenges and learning opportunities for all involved. Government agencies took on new and expanded roles and developed greater inter-agency participation, coordination, and information sharing. The months following the anthrax attacks stressed the system, but the system responded.

The field testing at the Brentwood P&DC trailer test facility was critical to determining the conditions required for large-scale remediation. Although these conditions were concluded with incomplete and less than perfect data, the conclusions were accurate as demonstrated by subsequent testing at the Beltsville, Maryland, test trailer and at the U.S. Army Dugway Proving Ground.

Clearly, the mechanism of action of chlorine dioxide gas on bacterial spores is not well understood, both at the molecular and operational levels. More extensive experimentation with greater number of experimental data points are required to clearly identify the optimal parameters for effective fumigation.

Some points are clear from all of the experimentation conducted and referenced in this report. Chlorine dioxide gas is effective at killing bacterial spores at relative humidity values in excess of 75 percent and at temperatures above 75°F, if applied for a total exposure of at least 9,000 ppmv-hours. The requirement for water is not fully understood but may facilitate the interaction between the spore coat proteins and the oxidative gas. The activities of the gas make it an attractive choice for the remediation of biological contamination on both small and large-scale operations.

It is evident that more training would have facilitated a smoother operation and analysis. The training for handlers of the spore strips, as well as training for the other operators as to the precautions to take in the presence of these indicator strips, was insufficient. Additional training for the laboratory analysis teams was also needed.

Lessons learned from this overall operation have been extensively documented and are extremely valuable. This report documents that the judgments made relative to the conditions for fumigation of the HSOB were supported by early field experimentation and supported by later laboratory experiments.

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