

SANDIA REPORT

SAND2006-3560
Unlimited Release
Printed June 2006

Bio-Restoration of Major Transportation Facilities Domestic Demonstration and Application Project (DDAP): Technology Development at Sandia National Laboratories

Richard O. Griffith, James L. Ramsey, Patrick D. Finley, Brad J. Melton, John E. Brockmann, Dan A. Lucero, Sean A. McKenna, Chad E. Peyton, Wayne Einfeld, Robert G. Knowlton, Pauline Ho, Gary S. Brown, Rita Betty, and Mark D. Tucker

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000.

Approved for public release; further dissemination unlimited.



Issued by Sandia National Laboratories, operated for the United States Department of Energy by Sandia Corporation.

NOTICE: This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government, nor any agency thereof, nor any of their employees, nor any of their contractors, subcontractors, or their employees, make any warranty, express or implied, or assume any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represent that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government, any agency thereof, or any of their contractors or subcontractors. The views and opinions expressed herein do not necessarily state or reflect those of the United States Government, any agency thereof, or any of their contractors.

Printed in the United States of America. This report has been reproduced directly from the best available copy.

Available to DOE and DOE contractors from
U.S. Department of Energy
Office of Scientific and Technical Information
P.O. Box 62
Oak Ridge, TN 37831

Telephone: (865) 576-8401
Facsimile: (865) 576-5728
E-Mail: reports@adonis.osti.gov
Online ordering: <http://www.osti.gov/bridge>

Available to the public from
U.S. Department of Commerce
National Technical Information Service
5285 Port Royal Rd.
Springfield, VA 22161

Telephone: (800) 553-6847
Facsimile: (703) 605-6900
E-Mail: orders@ntis.fedworld.gov
Online order: <http://www.ntis.gov/help/ordermethods.asp?loc=7-4-0#online>



SAND2006-3560
Unlimited Release
Printed June 2006

Biological Restoration of Major Transportation Facilities Domestic Demonstration and Application Project (DDAP): Technology Development at Sandia National Laboratories

Richard O. Griffith, James L. Ramsey, Patrick D. Finley, Brad J. Melton,
John E. Brockmann, Dan A. Lucero,
Plasma/Aerosol/Non-Continuum Processes Department

Sean A. McKenna, Chad E. Peyton,
Geohydrology Department

Wayne Einfeld, Robert G. Knowlton, Pauline Ho, Gary S. Brown,
Rita Betty, Mark D. Tucker
Chemical and Biological Systems Department

Sandia National Laboratories
P.O. Box 5800
Albuquerque, NM 87185

Abstract

The Bio-Restoration of Major Transportation Facilities Domestic Demonstration and Application Program (DDAP) is a designed to accelerate the restoration of transportation nodes following an attack with a biological warfare agent. This report documents the technology development work done at SNL for this DDAP, which include development of the BROOM tool, an investigation of surface sample collection efficiency, and a flow cytometry study of chlorine dioxide effects on *Bacillus anthracis* spore viability.

Acknowledgments

The authors (below) thank the Office of Research and Development at the U.S. Department of Homeland Security for funding this project.



We thank Duane Lindner and J. Bruce Kelley for their management support and Veronica Lopez for her administrative support of the project.

This work was performed at Sandia National Laboratories under a WFO agreement for the U.S. Department of Homeland Security. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

Contents

Abstract.....	3
Acknowledgments.....	4
Contents	5
Figures.....	6
Tables.....	8
1 Background.....	9
2 Project Overview	9
2.1 Restoration Process.....	9
2.2 Approach.....	12
2.3 Documentation.....	13
3 BROOM Software Tool.....	18
4 Joint Sandia/NIOSH Exercise.....	21
4.1 Introduction.....	21
4.2 Exercise Description	22
4.3 Results.....	23
4.4 User Recommendations	25
5 BROOM Demonstration at SFO.....	26
5.1 PDA Sample Collection.....	27
5.2 Characterization and Clearance Support.....	29
5.3 Decontamination Support	30
5.4 Sampling Design Tools.....	33
6 Surface Sample Collection Efficiency	37
6.1 Overview.....	37
6.2 Results.....	38
7 Evaluation of Spore Viability Analysis by Flow Cytometry	42
7.1 Overview.....	42
7.2 Results and Discussion	42
8 Summary and Conclusions	51

Figures

Figure 2-1. Stages of the restoration process, with time periods estimated from previous restoration activities.....	10
Figure 3-1. PDA replaces manual measurement and record-keeping tools.....	18
Figure 3-2. Desktop application displaying contours of contaminant concentration from statistical analysis.....	19
Figure 3-3. Data flow in BROOM tool.....	20
Figure 4-1. Aerial View of Coronado Club showing facility layout. North is to right edge of photo.	21
Figure 4-2. NIOSH team members in PPE collecting samples in Coronado Club.....	22
Figure 4-3. Sign in Coronado Club placed by NIOSH team.	23
Figure 4-4. DustTrak plot from yellow Visolite release.....	24
Figure 4-5. Broom contamination map (log10 scale) of basement using the 130 yellow Visolite wipe samples.....	25
Figure 5-1. Aerial photograph, internal view, and map showing the location of the BROOM demonstration at San Francisco International Airport.....	26
Figure 5-2. Samplers during BROOM demo at SFO.....	27
Figure 5-3. Hands-on demonstration of BROOM PDA application.....	27
Figure 5-4. Screen-shots from BROOM PDA. (a) Main menu. (b) Building map showing sample locations. (c) Details of sample location.	28
Figure 5-5. Screen-shots from BROOM PDA. (a) Sample bar code is read. (b) Pre-programmed sample types. (c) Initiation of chain-of custody report.....	28
Figure 5-6. BROOM is used to support characterization and clearance sampling.....	29
Figure 5-7. BROOM screenshot with building map, sample locations, air-handling zones.	29
Figure 5-8. Chain of Custody Report generated by BROOM	30
Figure 5-9. BROOM is used to support decontamination operations, including the placement and management of BIs during remediation.....	30
Figure 5-10. Example of a biological indicator.	31
Figure 5-11. BROOM screen shot showing several decon zones defined for a building.....	32
Figure 5-12. BROOM screen shot showing 2D and 3D maps.....	33
Figure 5-13. Statistical tools extract maximum information from limited sample datasets.	33
Figure 5-14. Sample design tools implemented in BROOM.....	34
Figure 5-15. Kriging creates estimated concentration map from a limited sample set.....	34
Figure 5-16. Contamination and uncertainty maps from BROOM tool.....	35
Figure 5-17. Recommended sample locations (triangles) from adaptive algorithm.....	35

Figure 6-1. Swabs, wipes and vacuum filter socks used for sampling.	37
Figure 6-2. Photos of aerosol test chamber and array of samples laid out for exposure.	38
Figure 6-3. Photos illustrating collection, extraction and recovery, left to right.	38
Figure 7-1. Germination rates for selected enhancement solutions.	43
Figure 7-2. Reduction in viable spores with time of exposure to chlorine dioxide: Red diamonds and curve: estimated values based on literature data. Green squares and curve: observed values.	44
Figure 7-3. Reduction in viable spores with time of exposure to chlorine dioxide: experimental values with and without use of neutralizer.	45
Figure 7-4. Reduction in viable spores with time of exposure to chlorine dioxide for BIs with three different spore concentrations.	46
Figure 7-5. Schematic of flow cytometer used for detection of vegetative cells.	46
Figure 7-6. <i>Bacillus atrophaeus</i> cells stained with Syto 9 fluorescent dye.	47
Figure 7-7. Comparison between flow cytometry and culture results.	47
Figure 7-8. Untreated <i>Bacillus atrophaeus</i> spores (left) and 30 minute chlorine dioxide treated <i>Bacillus atrophaeus</i> spores (right).	48
Figure 7-9. Syto 9 stained <i>Bacillus atrophaeus</i> cells after 5 hour fumigation.	49
Figure 7-10. <i>Bacillus atrophaeus</i> germinated cells stained with Syto 9 (green, viable) and propidium iodide (red, non-viable).	49

Tables

Table 6-1. Extraction and recovery efficiencies (η) for the swab method with polyester medium from stainless steel and painted wallboard surfaces	39
Table 6-2. Extraction and recovery efficiencies (η) for the wipe method with polyester/rayon medium from stainless steel and painted wallboard surfaces	39
Table 6-3. Extraction and recovery efficiencies (η) for the vacuum method with filter sock medium from stainless steel and painted wallboard surfaces	39
Table 6-4. Extraction and recovery efficiencies (η) for the vacuum method with filter sock medium from carpet and bare concrete surfaces.....	40
Table 6-5. Derived detection limits for characterization samples, where quantitative results are required.	40
Table 6-6. Derived detection limits for clearance samples, where only qualitative results are required.	41
Table 7-1. Estimated and observed spore log reduction times	44

Biological Restoration of Major Transportation Facilities Domestic Demonstration and Application Project (DDAP): Technology Development at Sandia National Laboratories

1 Background

The events of Fall 2001 demonstrated that the United States is not prepared to deal with the consequences of biological terrorism. Despite the increasing awareness of the threat and the potential impact of a release of biological agent, significant gaps exist in response and decision-making capabilities. These deficiencies were particularly evident with respect to the contamination of public and private facilities from letters containing *Bacillus anthracis* spores. The remediation of the Hart Senate Office Building, for example, took several months at a considerable cost before it was considered safe enough for reentry. Even now, there are still no widely accepted approaches for facility restoration.

The nation's inability to deal effectively with facility contamination raises the specter of severe social disruption and economic impact from future biological agent incidents. Biological contamination is especially a concern for many critical infrastructure elements. One class of facilities is particularly vulnerable: facilities and areas with high public traffic such as transportation nodes. Even with the enhanced security that is now present at many (but not all) transportation nodes these facilities remain highly vulnerable to a biological attack that could cause widespread contamination. Closure of a few key sites could have major consequences for the US economy. If even small amounts of *B. anthracis* spores were disseminated at four or five major hubs in the US, the world-wide air transportation system would be severely disrupted and recovery would be extremely slow because of the lack of widely accepted procedures and standards for decontamination and restoration.

2 Project Overview

The Bio-Restoration of Major Transportation Facilities Domestic Demonstration and Application Program (DDAP) is a collaborative project between Sandia National Laboratories (SNL) and Lawrence Livermore National Laboratory (LLNL) funded by the US Department of Homeland Security (DHS). The primary objective of this project is to develop a set of procedures, plans, and technologies for the rapid restoration of transportation nodes following an attack with a biological warfare agent, with a focus on major airports.

2.1 Restoration Process

Figure 2-1 shows the stages of the bio-restoration process, along with estimates of the lengths of the different stages, based on previous remediation activities. The time of the overall restoration operation is governed by the length of the combined activities. It is thus important to approach restoration as a system and to speed up all of the stages rather than focusing on just one or two.

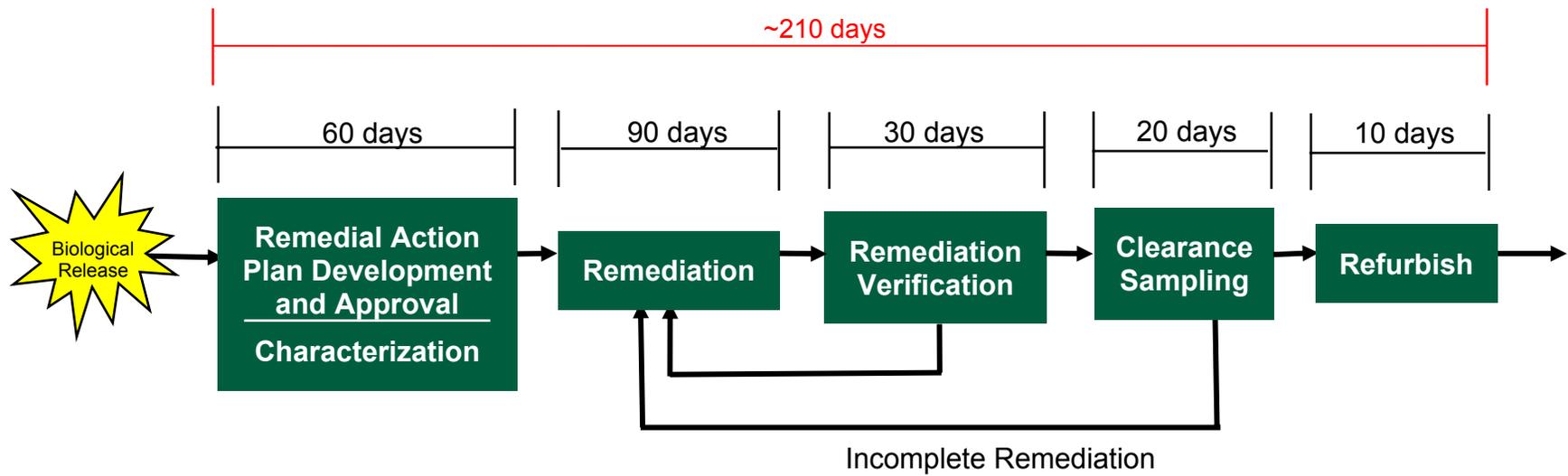


Figure 2-1. Stages of the restoration process, with time periods estimated from previous restoration activities.

The first stage of the restoration process, which would follow the efforts of the first responders, is the development and approval of the Remedial Action Plan and characterizing the extent of contamination. This stage can be fairly time-consuming and can require taking a large number of environmental samples. This stage involves:

- Develop sampling and analysis plan
 - Characterization
 - Clearance
- Develop decontamination plan
 - Clean-up goals
 - Approach for surfaces, spaces, sensitive equipment
 - Waste management
- Have plan approved by US EPA (FIFRA)
 - Crisis exemption must be granted
- Fully characterize extent and level of contamination
 - PCR or other techniques to determine the presence of the organism
- Identification of Technical Advisory Group and Clearance Committee

The next stage is the actual remediation and verification that the remediation technology worked. This stage can also be fairly time consuming, particularly if the remediation technology needs to be reapplied as a result of incomplete remediation. This stage involves:

- Conduct decontamination operations
 - Surfaces, spaces, sensitive equipment, waste
- Verify decontamination effectiveness
 - Biological indicators (spore strips)
 - Collection of Biological Indicators
 - Analysis of Biological Indicators (culturing)
- Waste management

The next stage in restoration is Clearance Sampling. This stage can require a very large number of samples to prove that the facility is safe to re-open. This stage involves:

- Environmental sampling to determine the presence of viable organisms
 - Targeted (areas of known contamination)
 - Biased (areas where contamination may accumulate)
 - Random/Grid
 - Laboratory analysis (culturing)
- Decision that facility is safe to re-open by the Clearance Committee and Facility Owner (i.e., have clean-up goals been met)

The last stage in the restoration process before re-occupying the facility is refurbishment. Except for the decision to re-open, which is likely to include input from other stakeholders, this is generally the responsibility of the facility owner. This stage often can be done in a timely manner, and involves activities such as:

- Repair and replace items removed or damaged during decontamination operations
 - Carpet
 - Chairs
 - Computers

- Test critical facility functions
 - Security
 - Safety
 - Operations
- Decision that facility is safe to re-open (as related to safety, security, and operations)

2.2 Approach

The DDAP project was designed to address the needs of all of the stages of the restoration process.

The first stage, Remedial Action Plan Development and Approval, is addressed by preparing, and getting pre-approval of, a “template” restoration plan for a major airport. The San Francisco International Airport (SFO) and other Bay area airports are used as representative facilities for this project. By conducting in-depth analyses of a limited number of facilities, this project was able to examine in detail many factors that must be considered in a restoration operation. From the facility-specific plans and procedures, a “template” will be extracted to simplify the development of restoration plans and procedures for a large number of similar facilities. The plans and procedures we developed in this project will fill a critical need in the near-term (next several years). It is well recognized that this subject is complex and that standards, protocols, and technologies for facility restoration will be evolving for years. These will be replaced by improved methods as better technologies become available and, possibly, as new standards are adopted. This plan is controlled distribution, and is available from LLNL to qualified requesters.

The Characterization, Remediation Verification, and Clearance Sampling stages of restoration all require large numbers of environmental samples. The process of obtaining, tracking, analyzing and managing such samples is a very time-consuming part of these stages. The DDAP therefore had several tasks to address aspects of sampling.

- SNL developed the Building Restoration Operations Optimization Model (BROOM) software. This system automates data entry, sample tracking, data management and spatial analysis. It is discussed in Section 3 of this document. Demonstrations of the capabilities of this tool under near-real-world conditions were done in Albuquerque NM (see Section 4) and at SFO (see Section 5).
- Surface sampling techniques such as swabs, wipes, and vacuum methods, are commonly used to determine the level of contamination. However, the efficiencies and thus the limits of detection for recommended sample collection methods are not well known for aerosol deposited biological agents. SNL work to resolve this issue is described in Section 6 of this document as well as in journal articles listed below.
- Analyzing the samples for the presence of the viable bio-agent can be rate-limiting. The current standard is to culture the samples, but this requires several days to get an answer and there is limited analysis capability in the US. Both LLNL and SNL worked on technologies for rapid determination of anthrax spore viability. LLNL work on a PCR methodology is described elsewhere. SNL work on a flow cytometry method is described in Section 7 of this document.

The Clean-up goals set in the Remedial Action Plan during the first stage, as well as the decision to re-open the facility during the Clearance and Refurbishment stages, depend on a decision as to

what “Clean” means in the case of a bio-agent. The ultimate judge of the effectiveness of a restoration operation is the traveling public through their willingness to re-occupy and use a restored facility, although it is likely that economic drivers will influence stakeholders to accept higher risks. The development of public standards or re-entry criteria must consider not only scientifically based information but also public perceptions about what is safe. Appropriate risk-communication techniques, including an explanation of scientific terminology and methods, should be used to implement recommendations based on scientific criteria. If the public is not convinced that a facility is safe, they will “vote with their feet.” For this reason, part of this project was to commission a nationally-recognized panel to re-examine the question of “How clean is safe?” to guide the process of acceptance of risks in the restoration of transportation nodes.

The project finished with a large-scale demonstration in January 2006 at SFO of the various technologies that were developed/assembled throughout the course of this project. The audience included the DHS sponsors as well representatives from many airports and transportation facilities. This kind of demonstration ensures that the plans and procedures can be executed effectively and are consistent with the capabilities and constraints of the organizations that would be responsible for operation. This exhibition also serves as a starting point for disseminating this remediation approach and its associated technologies to the many airports across the US that at this time have essentially no plans in place for bio-restoration activities. The overall goal of the project is to speed up the restoration process. An analysis of the time factors is described elsewhere.

2.3 Documentation

This report documents the technology development work done at SNL for this DDAP, as well as providing detailed information for some of the technology demonstrations. Much of the work is described in journal publications and other reports. In such cases, this document provides references to those works both here and in the appropriate sections below, rather than duplicating the technical material.

Journal Articles:

- *Bacillus Spore Recovery and Collection Efficiency for Non-Porous Surfaces Using Polyester Swabs.* G.S. Brown, R.G. Betty, et al. 2006. Manuscript in review for submission to Journal of Applied Microbiology.
- *Bacillus Spore Recovery and Collection Efficiency for Non-Porous Surfaces Using Polyester/Rayon Blend Wipes.* G.S. Brown, R.G. Betty, et al. 2006. Manuscript in review for submission to Applied and Environmental Microbiology.
- *Bacillus Spore Recovery and Collection Efficiency for Non-Porous Surfaces Using Vacuum Socks.* G.S. Brown, R.G. Betty, et al. 2006. Manuscript in review for submission to Environmental Science and Technology.
- *Low concentration chlorine dioxide sterilization of Bacillus atrophaeus spores.* G.S. Brown, , C.A. Souza, K.S. Walsh, R.M. Boucher, M.S. Tezak, and M.C. Wilson. 2006. Manuscript in preparation.
- *Rapid viability via PCR.* Staci Kane et al. In preparation.

Other Publications:

- *Reopening Public Facilities after a Biological Attack: A Decision Making Framework*, The National Academies Press, Washington, DC, 2005, ISBN 0-309-09661-8. Also available at: <http://www.nap.edu/books/0309096618/html> (May 2006).
- *Joint Sandia/NIOSH Exercise on Aerosol Contamination Using the BROOM Tool*, R. O. Griffith, et al., Sandia National Laboratories Report No. SAND2006-3784, June 2006.
- *User's Manual for the BROOM Software System*, James L. Ramsey, Patrick D. Finley, Brad J. Melton, Sean A. McKenna, Chad E. Peyton, and Christian A. Baugher, Sandia National Laboratories Report No. SAND2006-XXXX, in preparation.
- *Design and Implementation of the BROOM Software Package*, James L. Ramsey, Patrick D. Finley, Brad J. Melton, Sean A. McKenna, Chad E. Peyton, and Christian A. Baugher, Sandia National Laboratories Report No. SAND2006-XXXX, in preparation.
- *Analysis of Building Restoration Timeframes*. Ellen Raber and Mark Tucker. In preparation.

Presentations:

- *A Comparison of Room Concentration Fields Estimated via Kriging and Deterministic Airflow Models*, J.S. Bennett, S.A. McKenna, P. Finley, S. Shulman, W.K. Sieber, M. Katzoff, A. Wouhib, J. Brockman, and R. Griffith, presented at: International Biometric Society, Eastern North American Region (ENAR) Spring Meeting, Tampa, Florida, March 26th, 2006.
- *Mapping Contaminants in Buildings*, S.A. McKenna, and P.D. Finley, (invited presentation), First Annual Conference on Quantitative Methods and Statistical Applications in Defense and National Security, RAND Institute, Santa Monica, California, February 15-16, 2006.
- *BROOM: A Tool to Simplify the Collection and Analysis of Building Contaminant Data*, Patrick Finley, James Ramsey, Sean McKenna, Brad Melton Fall Technical Meeting of the American Industrial Hygiene Association, October 20, 2005, Albuquerque NM.
- *Using GIS Technology to Manage Information Following a Bioterrorist Attack*, Patrick Finley, Brad Melton, James Ramsey, ESRI Homeland Security GIS Summit, September 12-14, 2005, Denver CO.
- *Environmental Sampling Strategies Following a BioWatch Verified Positive*. G.S. Brown, North East Regional BioWatch Epidemiology Workgroup Conference, New Haven, CT, July 2005.

- *Evaluation of Surface Sample Collection Methods for Bacillus Spores on Porous and Non-Porous Surfaces.* G.S. Brown, West Regional BioWatch Epidemiology Workgroup Conference, Denver, CO, July 2005.
- *Environmental Sampling Strategies Following a BioWatch Verified Positive.* G.S. Brown, Midwest 1 Regional BioWatch Epidemiology Workgroup Conference, Chicago, IL, June 2005.
- *Environmental Sampling Strategies Following a BioWatch Verified Positive.* G.S. Brown, Texas Regional BioWatch Epidemiology Workgroup Conference, Dallas, TX, June 2005.
- *Evaluation of Surface Sample Collection Methods for Bacillus Spores on Porous and Non-Porous Surfaces.* G.S. Brown, EPA Environmental Sample Collection Workshop, San Diego, CA, May 2005.
- *Environmental Sampling Strategies Following a BioWatch Verified Positive.* G.S. Brown, EPA Environmental Sample Collection Workshop, San Diego, CA, May 2005.
- *Evaluation of Surface Sample Collection Methods for Bacillus Spores on Porous and Non-Porous Surfaces.* G.S. Brown, BioWatch Epidemiology Workgroup Planning Meeting, Austin, TX, May 2005.
- *Contamination Characterization in Buildings – A Spatial Statistics Based Approach,* Sean A. McKenna, James Ramsey, Chad Peyton, Patrick Finley, Brad Melton, Richard Griffith, (poster), DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Restoration of Major Transportation Facilities Following a Chemical Agent Release,* Mark D. Tucker, DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Source Term Dose Response Analysis Toolset,* John E. Brockmann, Fred Harper, Douglas Sommerville, DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Restoration of Major Transportation Facilities Following a Biological Agent Release,* Richard O. Griffith, DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Evaluation of Surface Sample Collection Methods for Bacillus Spores on Porous and Non-Porous Surfaces,* Gary S. Brown, DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005..
- *Rapid Recovery Using the Building Restoration Operations Optimization Model,* James Ramsey, Patrick Finley, Brad Melton, Sean McKenna, Chad Peyton, Proceeding of the DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.

- *DF-200, An Enhanced Sandia Decontamination Formulation for Neutralization of CBW Agents and Toxic Industrial Chemicals*, Mark D. Tucker and Rita G. Betty, (poster), DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Spore Viability Determination by Rapid Germination and Flow Cytometry Detection* Gary S. Brown, (poster), DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Rapid Inactivation and Verification of High-Consequence Agricultural Viral Agents*, Wayne Einfeld, DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Incorporating Building Geometry into Estimates of Contaminant Levels*, Patrick Finley and Sean McKenna, (poster) Proceedings of the DHS-IEEE Working Together: Research and Development Partnerships in Homeland Security Conference, Boston, MA, April 27-28, 2005.
- *Evaluation of Current CDC Recommended Surface Sample Collection Method for Dry Deposited Bacillus Spores on Porous and Non-Porous Surfaces*. G.S. Brown, Decon Downunder, Melbourne, Australia, February 2005.
- *Rapid Recovery Using the Building Restoration Operations Optimization Model*, J. Ramsey, P. Finley, B. Melton, R. Griffith, S.A. McKenna, C. Peyton, First Annual National Conference on Environmental Sampling for Bio-Threat Agents, Baltimore, Maryland, Jan. 27-28, 2005.
- *Contamination Characterization in Buildings – A Spatial Statistics Based Approach*, Sean A. McKenna, James Ramsey, Chad Peyton, Patrick Finley, Brad Melton, Richard Griffith, (oral and poster presentations), First Annual National Conference on Environmental Sampling for Bio-Threat Agents, Baltimore, Maryland, Jan. 27-28, 2005.
- *Evaluation of the Wipe Surface Sample Collection Method for Dry Deposited Bacillus Spores on Non-Porous Surfaces*, Gary S. Brown, Rita Betty, Raymond M. Boucher, Daniel A. Lucero, Caroline A. Souza, Matthew S. Tezak, Kathryn S. Walsh, Mollye C. Wilson, First Annual National Conference on Environmental Sampling for Bio-Threat Agents, Baltimore, Maryland, Jan. 27-28, 2005.
- *Aerosol Sampling Issues: The Case for Application-Specific Sampling Inlet Design and Calibration*, John E. Brockmann, Daniel A. Lucero, Todd Rudolph, Jennifer Olson, First Annual National Conference on Environmental Sampling for Bio-Threat Agents, Baltimore, Maryland, Jan. 27-28, 2005.
- *Aerosol Deposition Chamber*, John E. Brockmann, Daniel A. Lucero, Todd Rudolph, Jennifer Olson, (poster), First Annual National Conference on Environmental Sampling for Bio-Threat Agents, Baltimore, Maryland, Jan. 27-28, 2005.
- *Sampling Hazardous Materials Using Recent Developments in Positioning Technology*, Patrick Finley, James Ramsey, Brad Melton, Jay Cadman (Ubisense),

(poster), First Annual National Conference on Environmental Sampling for Bio-Threat Agents, Baltimore, Maryland, Jan. 27-28, 2005.

Workshop Proceedings:

Proceedings from workshops and meetings that were held as part of the DDAP were distributed to participants and other parties with a demonstrated interest in restoration planning. The proceedings are designated as Official Use Only (OUO). They are listed below and are available to qualified requestors from LLNL by referring to the UCRL publication number.

- *Proceedings of the Restoration Workshop*, S. Mancieri, R. Kirvel, M. Tucker, and E. Raber, Eds., Lawrence Livermore National Laboratory, Livermore, CA, UCRL-PROC-200943 (September 2003).
- *Proceedings of the Biological Restoration Tabletop Exercise*, S. Mancieri, R. Kirvel, M. Tucker, and E. Raber, Eds., Lawrence Livermore National Laboratory, Livermore, CA, UCRL-PROC-206018 (April 2004).
- *Proceedings of the LAX Biological Response Coordination Workshop*, S. Mancieri and R. Kirvel, Eds., Lawrence Livermore National Laboratory, Livermore, CA, UCRL-PROC-211908 (February 2005).
- *Proceedings of the SFO Biological Response Coordination Workshop*, S. Mancieri and R. Kirvel, Eds., Lawrence Livermore National Laboratory, Livermore, CA, UCRL-PROC-212577 (March 2005).
- *Proceedings of the SEA-TAC Biological Response Coordination Workshop*, S. Mancieri and R. Kirvel, Eds., Lawrence Livermore National Laboratory, Livermore, CA, UCRL-PROC-212775 (March 2005).
- *Preparing for Bio-Contamination of an Airport Facility*, S. Mancieri, D. Canter, E. Raber, R. Kirvel, and J. Kempter, Eds., Lawrence Livermore National Laboratory, Livermore, CA, UCRL-PROC-217654 (June 2005).
- *Proceedings of the Facility Biological Restoration Technology Demonstration Held at San Francisco International Airport*, E. Raber and M.D. Tucker, Program Mgrs., UCRL-PROC-XXXXXX (June 2006)

3 BROOM Software Tool

James L. Ramsey, Patrick D. Finley, Brad J. Melton

The Building Restoration Operations Optimization Model (BROOM) is a software product developed to assist in the restoration of major transport facilities in the event of an attack involving chemical or biological materials. As shown in Figure 3-1, the objective of this work is to replace a manual, paper-based data entry and tracking system with an electronic system that should be much less error-prone. It will also manage the sampling data efficiently and produce contamination maps in a more timely manner.

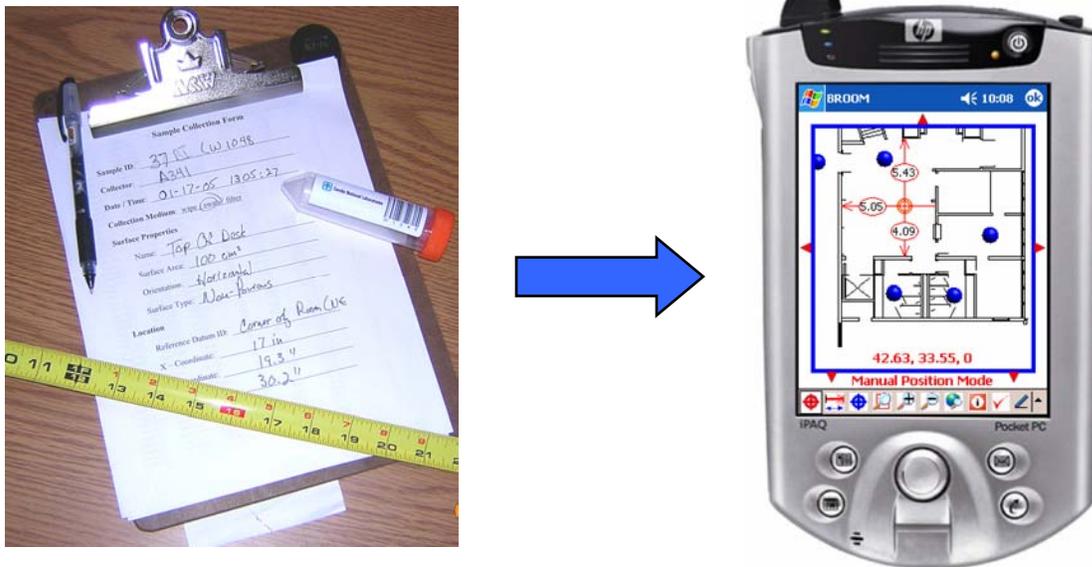


Figure 3-1. PDA replaces manual measurement and record-keeping tools.

The BROOM software consists of two independent but interfacing applications. The first application runs on a handheld Pocket PC and is designed to collect and record surface sampling data during the characterization and verification phases of decontamination. The device may also be used to record the position of biological indicators prior to fumigation in the decontamination phase. The handheld device is optionally equipped with a commercially available barcode scanner and wireless laser range finder. The barcode scanner provides a means of uniquely identifying and tracking samples from the point of origination through the laboratory analysis process. The laser range finder is used to precisely define the location of the sampled surface with respect to interior structures of the building. Additional data, such as the properties of the sampling surface, sample type, date, and time, are also recorded effortlessly. The handheld device can be assembled for about \$1800 at the time of this writing, or a ruggedized version for ~\$3200.

The second application runs on a Windows desktop platform and serves to manage, analyze, and visualize sampling results (Figure 3-2). The desktop application works in conjunction with an SQL Server database to store, retrieve, visualize, and analyze the laboratory results of sampling activities. The database design is significant in that it allows simultaneous multiple user access to sample data. Building floor plans and other pertinent drawings of interest are organized by floor and also stored in the database. Database storage is also a key component of the modular software design. Analysis tools can be added relatively quickly to BROOM using generic procedures that retrieve inputs and write outputs to the database. Furthermore, the outputs from one tool are then readily available as inputs to additional tools.

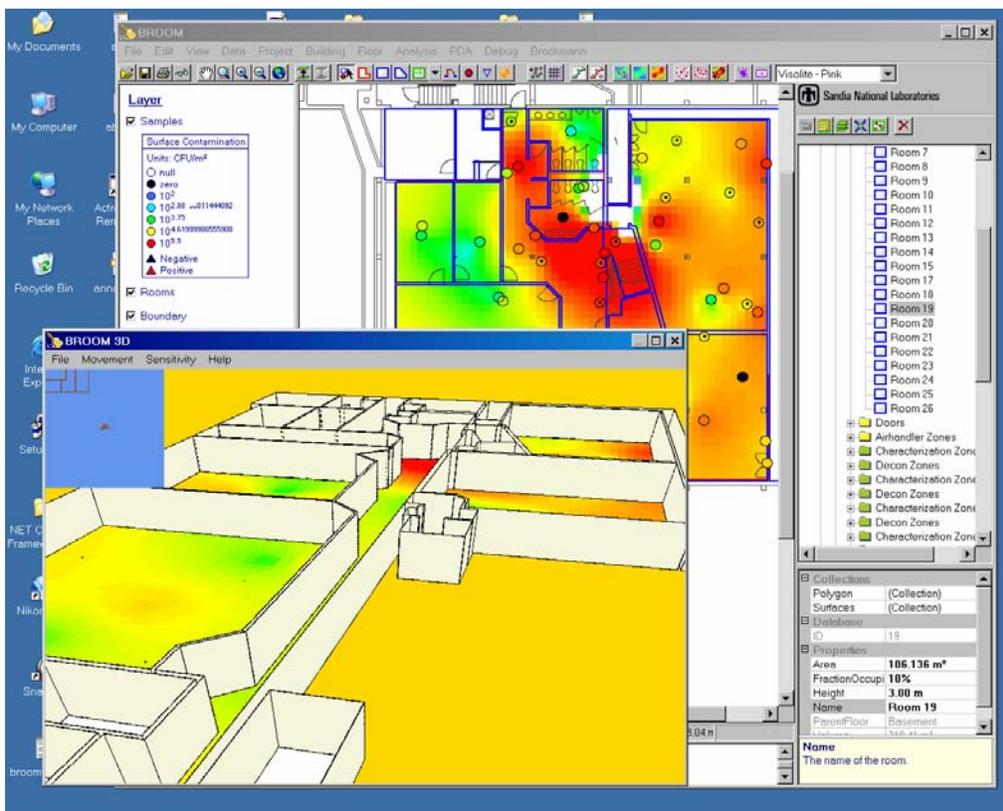


Figure 3-2. Desktop application displaying contours of contaminant concentration from statistical analysis.

An overview of the BROOM data flow is presented in Figure 3-3. Building floor plans and proposed sampling locations can be downloaded from a BROOM equipped computer located either in clean area inside the building or at a safe distance outside the building to the handheld device over a wireless network. The data collected during sample acquisition are temporarily stored in the handheld device and upon completion, transmitted back to the computer over the same wireless network. The now contaminated handheld device may be left in the building to be fumigated with the rest of the building or inserted in a cradle to charge the batteries for future sampling efforts.

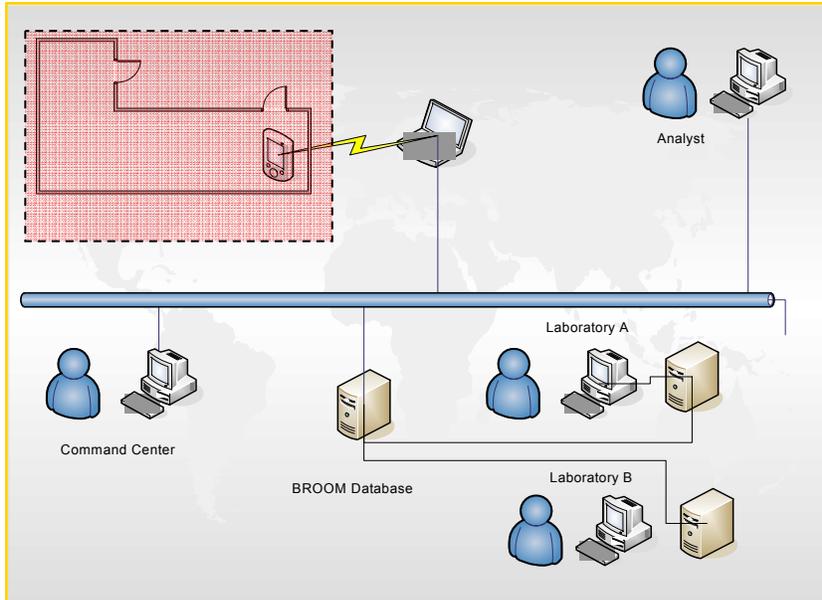


Figure 3-3. Data flow in BROOM tool.

The data analysis and visualization capabilities of BROOM are further described in the sections on the Coronado Club and SFO demonstration (sections 4 and 5). These demonstrations illustrate how the tool would be used in near-real-life cases, so we do not duplicate such descriptions here.

This software is in the process of being commercialized. User documentation is available as Sandia National Laboratories Reports (Ramsey, et al, 2006a,b)

References

Ramsey, James L., Patrick D. Finley, Brad J. Melton, Sean A. McKenna, Chad E. Peyton, and Christian A. Baugher, 2006a, *User's Manual for the BROOM Software System*, Sandia National Laboratories Report No. SAND2006-XXXX, in preparation.

Ramsey, James L., Patrick D. Finley, Brad J. Melton, Sean A. McKenna, Chad E. Peyton, and Christian A. Baugher, 2006b, *Design and Implementation of the BROOM Software Package*, Sandia National Laboratories Report No. SAND2006-XXXX, in preparation.

4 Joint Sandia/NIOSH Exercise

4.1 Introduction

A joint Sandia/NIOSH exercise was conducted in Albuquerque, NM February 16–25, 2005, at the Coronado Club (Figure 4-1), a closed SNL facility. This exercise and the results from it are described in detail in Sandia National Laboratories report SAND2006-3784, so we only include a brief discussion here.

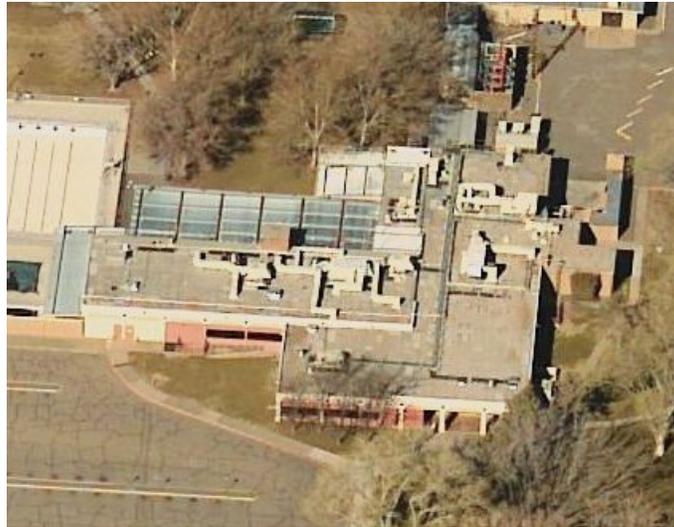


Figure 4-1. Aerial View of Coronado Club showing facility layout. North is to right edge of photo.

Both NIOSH and SNL had specific objectives for the exercise.

BROOM team objectives included:

- demonstration of the BROOM sample management tool under “real life” conditions by experienced sample collection teams,
- demonstration of the BROOM contamination mapping module,
- demonstration of the BROOM sampling strategy planning tool, and
- development of an actual surface contamination database following a tracer aerosol release for evaluation of statistical algorithms.

NIOSH team objectives included:

- demonstration of mobile sampling deployment capabilities,
- demonstration of semi-automated sample logging hardware (ruggedized PC-tablet), and
- evaluation of onsite decontamination procedures for removal of a tracer aerosol contamination.

All objectives were met for both the SNL BROOM team and the NIOSH team.

4.2 Exercise Description

A fluorescent-tagged tracer aerosol was used as a bioaerosol stimulant. The median particle diameter of the tracer aerosol was approximately 1 micrometer which is roughly comparable to a bacterial spore. Two variations of the aerosol tracer were used during the exercise. A yellow fluorescent variant of the tracer aerosol was released prior to the arrival of the NIOSH team, extensively sampled, and quantitatively analyzed. The analytical results were used not only to generate a detailed contamination distribution map for the facility but are also being used for the further development of statistical sampling algorithms. A pink fluorescent variant of the tracer aerosol was released from the same location as the mapping release the day before the sample collection teams entered the facility. All samples collected were analyzed for both pink and yellow aerosol tracer.

Two NIOSH teams collected samples from facility surfaces while in level C PPE (Figure 4-2). On the first day, one team collected surface samples in the morning and the other collected samples in the afternoon. On this initial entry, both teams selected sample locations based on expert judgment. All samples were analyzed overnight and concentration data supplied to both teams prior to a second day entry. The NIOSH team performed and evaluated personnel decontamination procedures for each sample collection team as they exited the facility following a sample collection effort. Decontamination effectiveness was evaluated following treatment using visual inspection with a UV light that caused any residual aerosol simulant to fluoresce.



Figure 4-2. NIOSH team members in PPE collecting samples in Coronado Club.

On the second day, a NIOSH team entered the facility in the morning and collected additional samples based on information and guidance provided by the BROOM tool. The second team generated contamination maps and additional sample locations by hand based on analytical data

and expert judgment, and then entered the facility for an afternoon sample collection effort. All samples were analyzed overnight and concentration data supplied to both teams prior to a third day entry.

The third day mapping and sample collection activities were conducted as on day 2. By the end of the day, the NIOSH expert judgment sample collection team had correctly identified the source location of the release (Figure 4-3).



Figure 4-3. Sign in Coronado Club placed by NIOSH team.

4.3 Results

The Visolite powder releases were characterized by an array of DustTraks and aerodynamic particle sizer (APS) measurements. DustTraks are portable laser-photometer instruments that measure and record airborne aerosol concentrations. The APS measures aerodynamic particle size and relative light scattering intensity. It will detect and measure particles in the size range of 0.5 to 20 micrometers in diameter.

Figure 4-4 shows a example of DustTrak results for the release of yellow Visolite powder in the Coronado Club. These results show that the aerosol cloud moved up the stairs from the basement release point quite quickly. The concentration remained high for about a half an hour before declining by a combination of mixing of clean air, and deposition of the particles on surfaces. Many other results, along with the experimental details, are given in SAND2006-3784.

Yellow Visolite Tracer Release
Feb 16, 2005

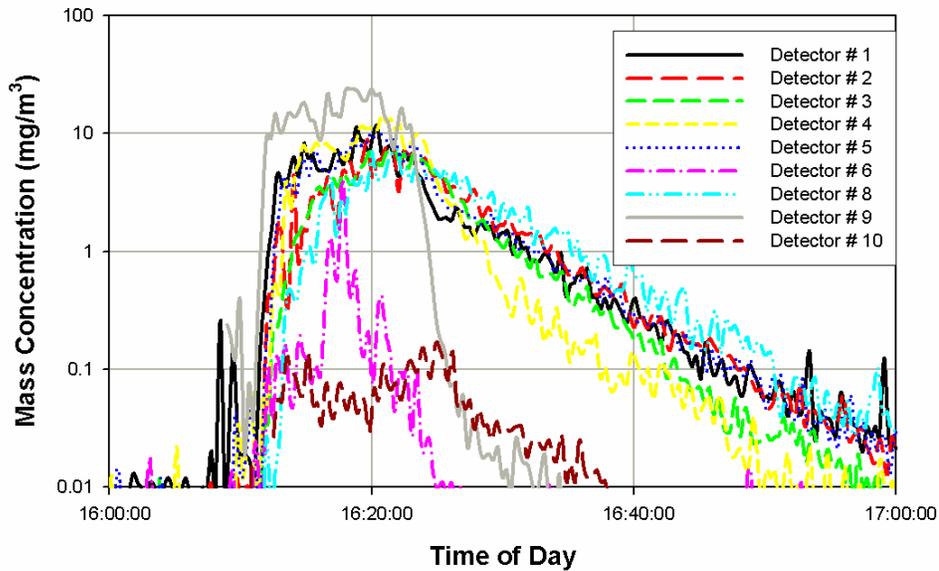


Figure 4-4. DustTrak plot from yellow Visolite release.

Figure 4-5 shows an example of a contamination map obtained by the ordinary kriging process in BROOM using the 130 yellow Visolite powder data set (wipes). The results clearly show the path of the yellow Visolite tracer from the release point in the nearly square conference room on the left side of the basement, out the door in the northeast corner of that room and north along the hallway to the base of the staircase. Many other results from statistical analyses of the results, as well as the numerical results themselves, are given in SAND2006-3784.

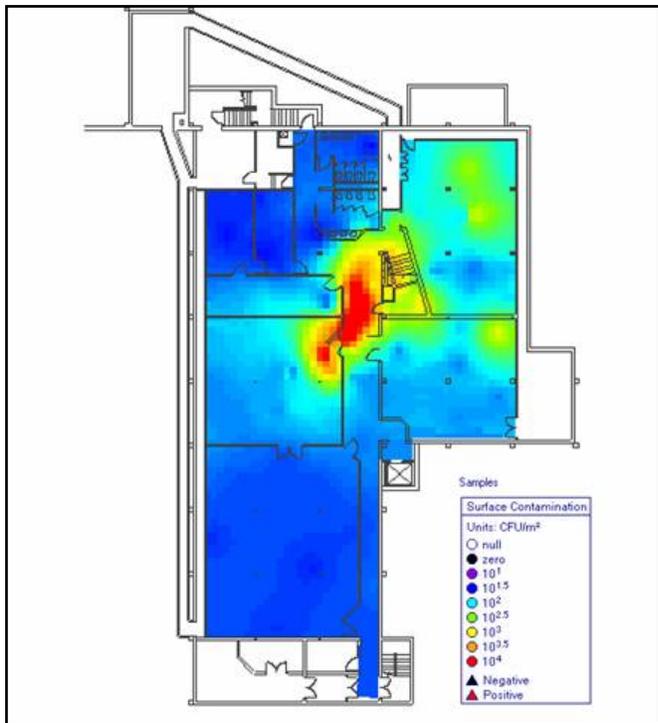


Figure 4-5. Broom contamination map (log₁₀ scale) of basement using the 130 yellow Visolite wipe samples.

4.4 User Recommendations

The expert users saw a number of advantages to using the BROOM tool in sampling:

- Small size, low weight of PDA.
- Rapid generation of contamination maps to summarize and communicate information. Getting information out in a usable form was important in the Anthrax responses.
- Real-time, electronic record, less error-prone than paper records.
- Ability to click on sample and see collection/location info, rather than paging through many sheets of paper.
- Valuable data management tool.
- Good for a major response, where need for large number of sample takers may mean that many of them are not experienced industrial hygienists.

In particular, although the BROOM tool was originally developed to assist in sampling, they thought that it would be very useful as a data management tool. The users had a number of specific suggestions for improvements, but were enthusiastic about being beta testers.

References

Joint Sandia/NIOSH Exercise on Aerosol Contamination Using the BROOM Tool, R. O. Griffith, et al., Sandia National Laboratories Report No. SAND2006-3784, June 2006.

5 BROOM Demonstration at SFO

The final project exhibition at SFO on January 25-26, 2006 included a demonstration of the BROOM tool in action. It was held in a part of the now-closed old international terminal building that was adjacent to where the lectures were held, shown in Figure 5-1.

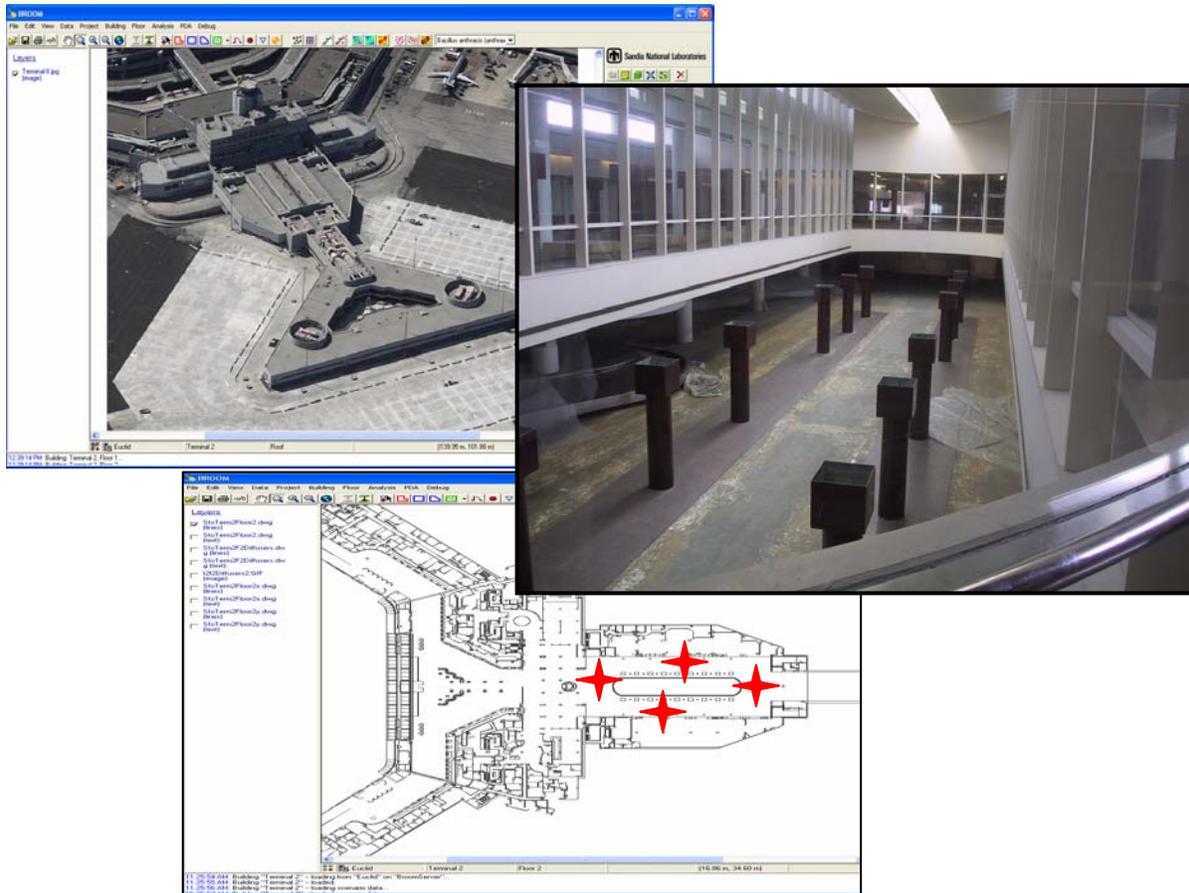


Figure 5-1. Aerial photograph, internal view, and map showing the location of the BROOM demonstration at San Francisco International Airport.

Four stations were set up in the upstairs portion of the building, roughly located at the ★ symbols on the map. The audience was divided into four groups and rotated through focused presentations given at these stations. The presentations are described below. On the floor below, sampling teams were collecting surface samples and placing biological indicators (BIs) (Figure 5-2). At appropriate times, wireless data transfers were done from the PDAs being used downstairs to the laptop computers being used by the BROOM demonstrators upstairs.



Figure 5-2. Samplers during BROOM demo at SFO.

5.1 PDA Sample Collection

This presentation (Figure 5-3) was a hands-on demonstration of how to use the PDA for sample collection, including use by a member of the audience. Sample collection occurs during the Characterization, Remediation Verification, and Clearance stages of the building restoration process.



Figure 5-3. Hands-on demonstration of BROOM PDA application.

The main menu for the PDA application is shown in Figure 5-4(a). “Take Samples” would be the main option used during Characterization or Clearance stages of restoration, while the “Deploy BIs” option would be used during the Remediation Verification stage. The “Download” option would be used to put building maps or new sets of suggested sample locations into a given PDA. The “Upload” option would be used periodically to transmit newly acquired positions to the desktop application, where they would immediately appear on the BROOM map, along with the current location of the sampling team. The “Set Collector” option would be used at the beginning of the sampling session or whenever the operator changed, while the “Set Floor” option ensures that the correct building map is being displayed, and would be used whenever the sampling team moved to a new floor.

Figure 5-4 (b) is a screen shot showing a building map with 4 suggested sample locations (blue dots) and the current location of the sampling team (red crosshairs). In this case, the PDA is in “Manual Position” mode, so the user has input their position by clicking on the map to place red crosshairs. In “Laser Position” mode, the user would click on the image of wall on the PDA to fire the laser rangefinder and measure their position. These features allow the sampling team to quickly navigate to the desired sample locations. Figure 5-4 (c) shows the “Sample Position” screen. The sample elevation above the floor (Z coordinate) is manually entered by the user.

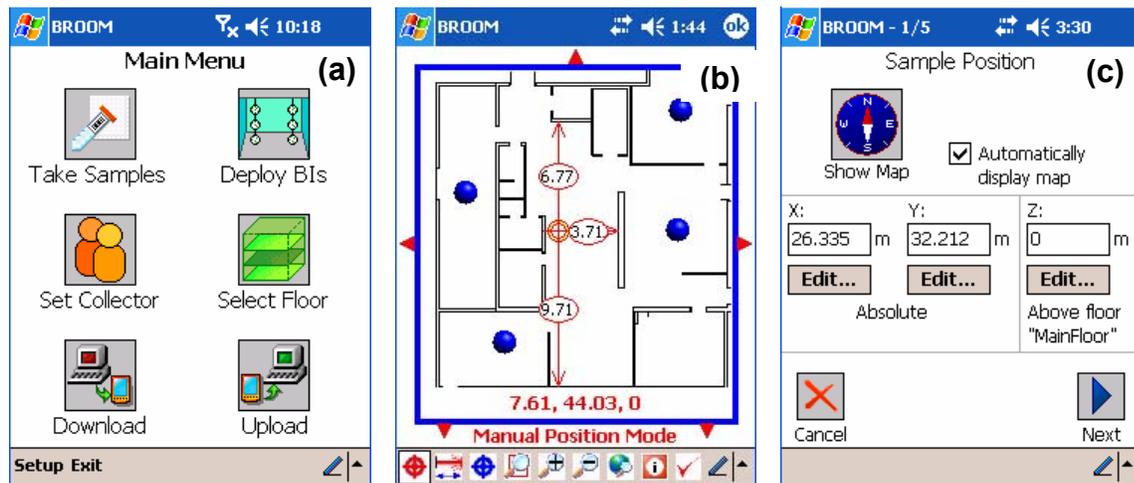


Figure 5-4. Screen-shots from BROOM PDA. (a) Main menu. (b) Building map showing sample locations. (c) Details of sample location.

Figure 5-5 (a) is the “Sample Bar Code” screen. Clicking the “Scan” button fires the barcode scanner; the resulting bar code value is displayed and stored in the PDA. Figure 5-5 (b) shows the types of samples pre-programmed into BROOM. The information on the type of sample is used in data analysis to correct for sampling efficiencies. Figure 5-5 (c) is the “Upload to Server” screen. This transmits the set of sampling information to the desktop application. The collector signs to initiate an Electronic chain-of-custody process.

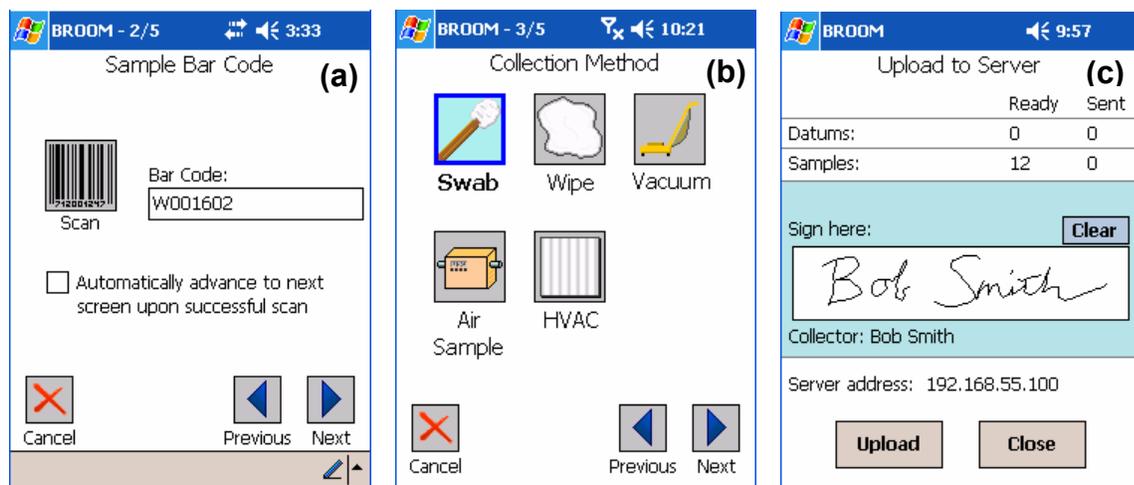


Figure 5-5. Screen-shots from BROOM PDA. (a) Sample bar code is read. (b) Pre-programmed sample types. (c) Initiation of chain-of-custody report.

5.2 Characterization and Clearance Support

This presentation (Figure 5-6) covered BROOM support of the Characterization (pre-cleanup) and Clearance (post-cleanup) stages of the restoration process. During Characterization, the goal is to determine the extent and magnitude of contamination, while in Clearance, the goal is to demonstrate that the facility is clean and safe for reentry. Both stages require taking large numbers of environmental samples, which would be greatly assisted by BROOM.



Figure 5-6. BROOM is used to support characterization and clearance sampling.

In planning for a restoration operation, preloading drawings and images into BROOM (Figure 5-7) can help analyze the HVAC system and define air-handling zones. During sample collection, BROOM would provide real-time guidance and monitoring via the wireless data transfer, as well as providing chain-of-custody reports (Figure 5-8) for the sampling teams. BROOM also assists in data management and visualization by providing a secure remote database, rapid access to large numbers of samples/analyses, and graphical maps to aid in interpreting the results.

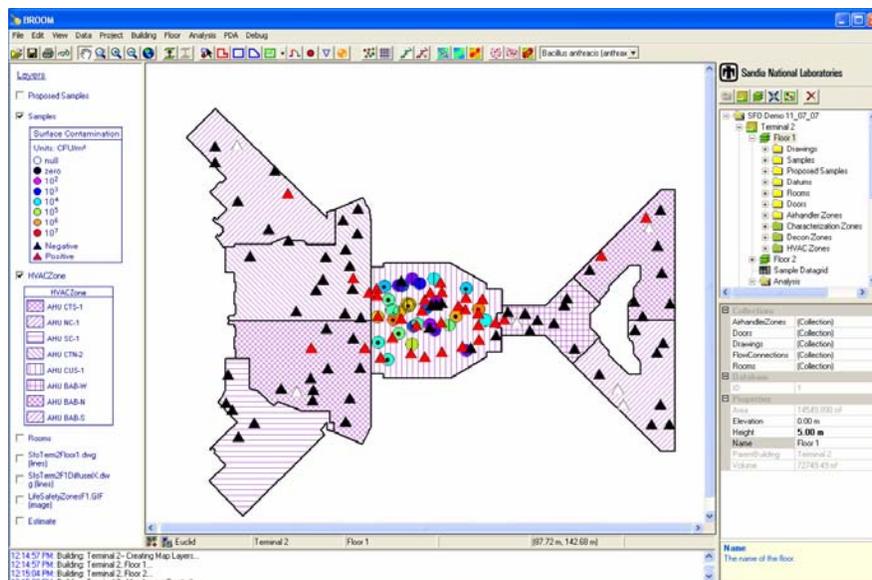


Figure 5-7. BROOM screenshot with building map, sample locations, air-handling zones.

During the remediation process, biological indicators or spore strips, Figure 5-10, are used to verify that the fumigation technology worked correctly. These are placed in the region to be treated at a density of roughly 1 BI per 100 ft² of contaminated area. A large building might require the placement and analysis of thousands of BIs to adequately characterize the effectiveness of the remediation. For each BI, one needs to track the location, time of collection, analytical results, and how that result compares with that of neighboring locations. These capabilities are provided by BROOM. The PDA also assists in the placement of the BIs. Desired locations for BIs can be transmitted to PDAs in the contaminated zone, where the placement teams can use floor plan in the PDA to quickly find the desired BI hanging site. The barcode would then be scanned to establish the link between the BI and the position. Automating this repetitive data entry task should reduce errors.



Figure 5-10. Example of a biological indicator.

Decontaminating a building generally requires dividing the building into zones and decontaminating a zone at a time. The size of a decon zone depends on the decon technology being used, but the best way to define a decon zone depends on the building structure and air handling system. Zones can be defined based on building schematics that have been loaded into the software, or user-drawn.

Figure 5-11 is a screenshot from the BROOM desktop application that shows several decon zones defined for a building. As shown, a zone can be named for a specific sub-unit of facility, and comments about the zone can be stored in an easily accessible manner. The software also tracks the following information about each zone:

- Area and volume
- Zone description
- Decon process
- Decon validation textual information
- Decon rating

Figure 5-12 is a BROOM screenshot showing a 2D contamination map and a 3D visualization. The latter is important in identifying samples with identical X and Y coordinates. These might be a string of BIs, or a surface samples from a ceiling light fixture, tabletop, and floor.

BROOM also provides a number of statistical tools to support the decision-making process. These are described in the next section.

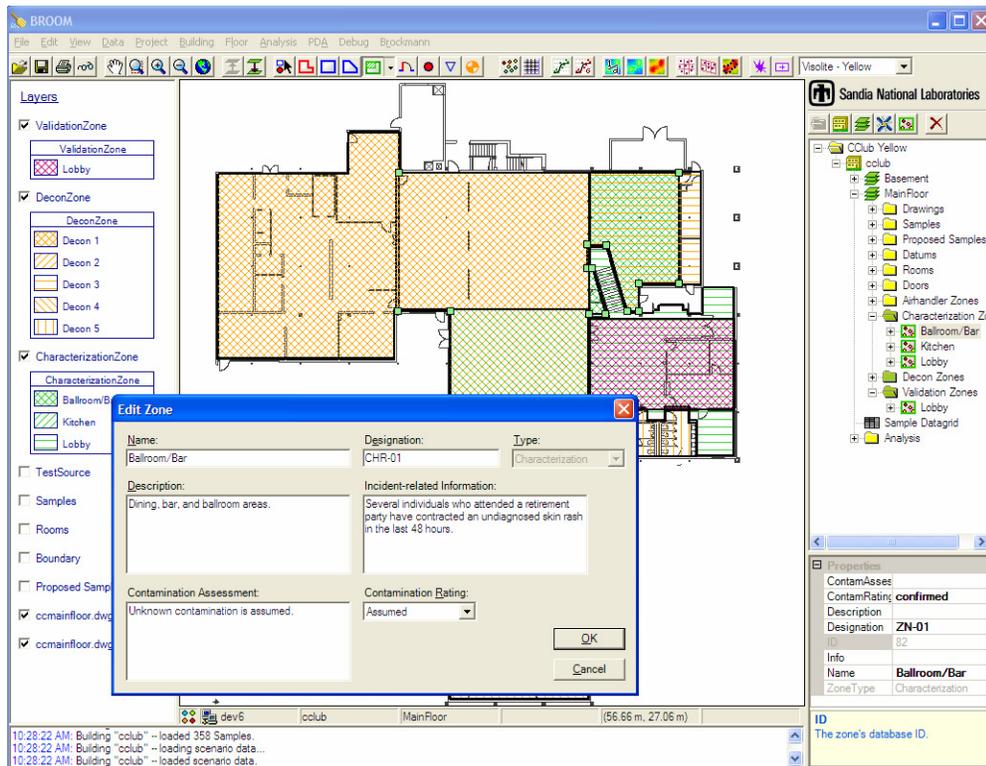


Figure 5-11. BROOM screen shot showing several decon zones defined for a building.

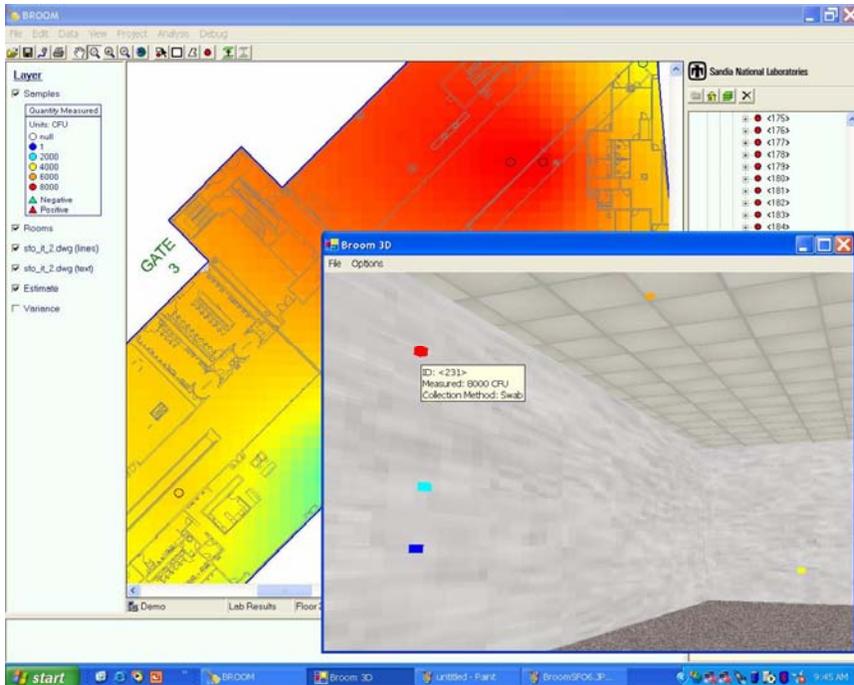


Figure 5-12. BROOM screen shot showing 2D and 3D maps.

5.4 Sampling Design Tools

This presentation (Figure 5-13) covered the use of BROOM to help decide where to collect samples and when to stop collecting them. Sample collection occurs during the Characterization, Remediation Verification, and Clearance stages of the building restoration process, and can be a substantial, time-consuming effort.

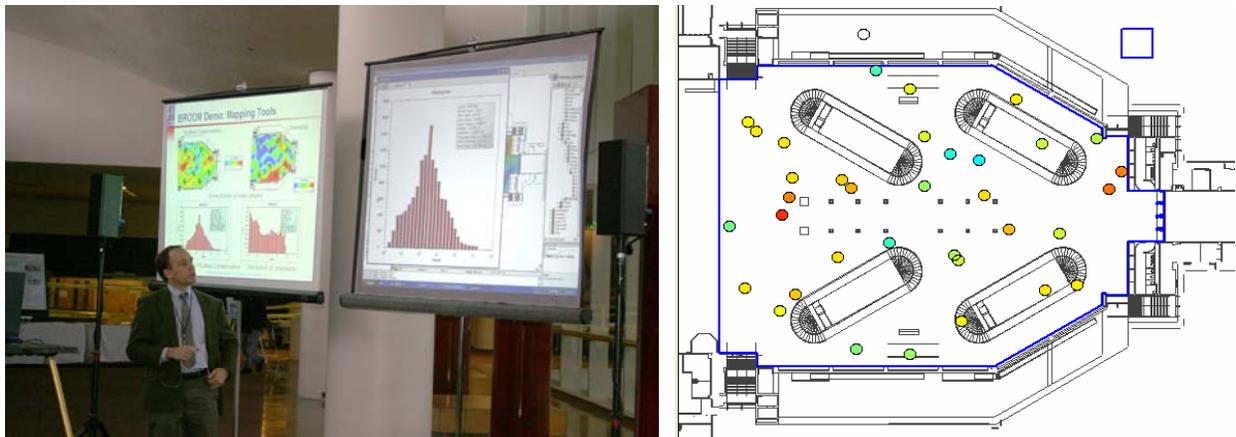


Figure 5-13. Statistical tools extract maximum information from limited sample datasets.

The work on sample design to date has focused on the characterization stage. The emphasis is on helping to decide where to take samples in order to define the extent and magnitude of contamination and thus provide a basis for decontamination design.

Several software options are available within BROOM for creating the sampling designs needed to support statistically-based characterization goals. As shown in Figure 5-14, the user can lay out a geometrically gridded, a random, or a stratified random sampling design on the building drawing. The EPA and MARSSIM guidance documents (EPA 1997, 2000) were used as basis for choosing these sampling designs.

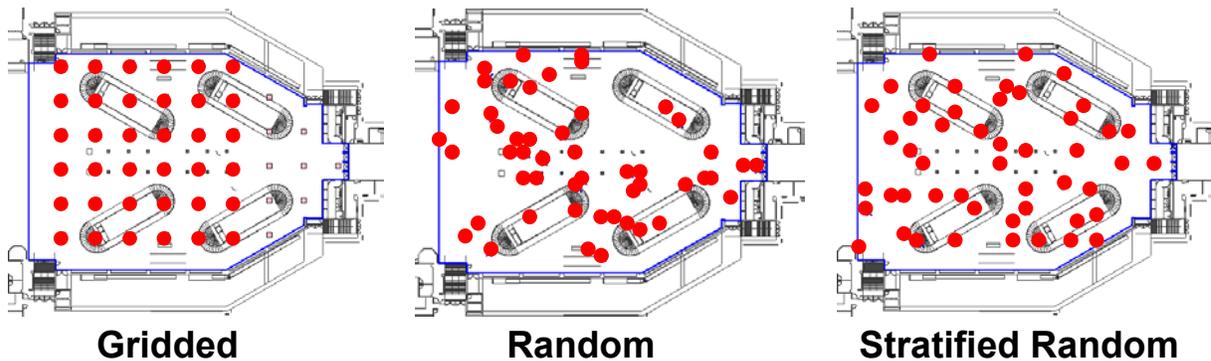


Figure 5-14. Sample design tools implemented in BROOM.

The sampling designs shown above would be best used in an initial round of sampling at the start of the characterization process. For subsequent sampling rounds, BROOM provides tools for adaptive sampling, where knowledge gained from previous round(s) of sampling are used to optimize the locations of the next round of samples. This is done using a geostatistical algorithm known as Kriging (Isaaks and Srivastava, 1989). As shown in Figure 5-15, this provides a map of estimated contamination magnitudes at all unsampled locations based on limited sample data and a model of spatial covariance. The assumption is that samples taken close together will be more similar than those taken far apart. Kriging also provides the uncertainty in that estimate at all unsampled locations.

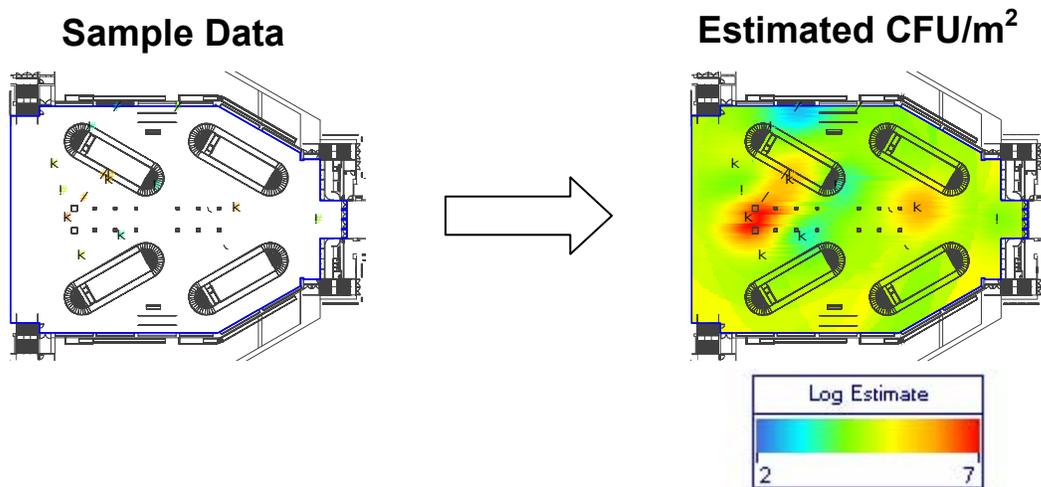


Figure 5-15. Kriging creates estimated concentration map from a limited sample set.

Figure 5-16 shows surface contamination and uncertainty maps created with 30 initial samples. This is used as input to an optimization algorithm to locate the next round of 15 samples. The

optimization objectives are to reduce the uncertainty in the estimated contamination values and identify the edges of the contamination hot spots. Adapting the sampling design should maximize the understanding (magnitude and extent) of the contamination and minimize the number of samples required, thereby speeding up and reducing the cost of the restoration.

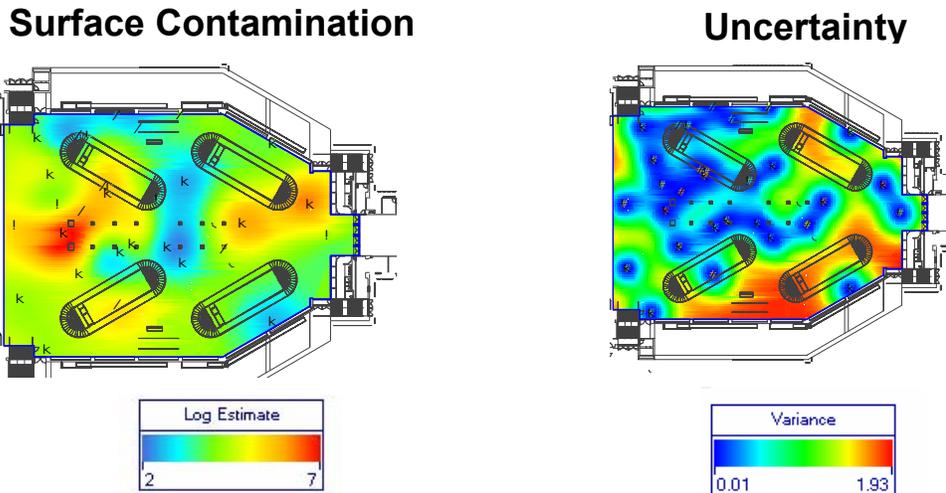


Figure 5-16. Contamination and uncertainty maps from BROOM tool.

Figure 5-17 shows the 15 proposed sample locations (triangles) that resulted from the adaptive sampling algorithm, on top of the estimated contamination map. These locations were optimized both to identify the extent of areas with elevated concentration, “hot spots” (30%) and to reduce uncertainty in distribution of contaminant (70%).

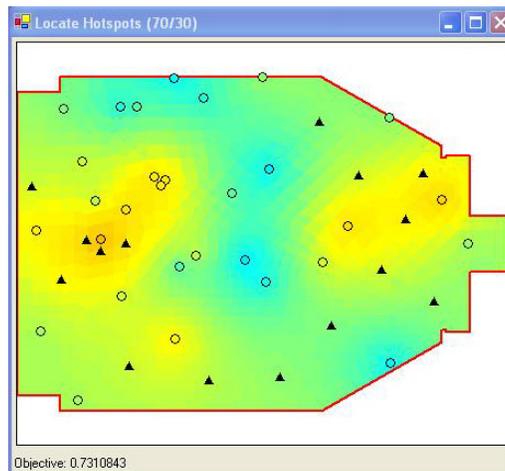


Figure 5-17. Recommended sample locations (triangles) from adaptive algorithm.

For post-decontamination clearance, the issue in sample design is deciding how many samples are needed to provide a defensible basis for reoccupation of building. This means figuring out how to efficiently achieve the requested confidence levels. In clearance sampling, the samples

are only tracked for positive or negative results based on detection limit of the analysis method. This contrasts with characterization sampling, where quantitative results are tracked in order to map the contamination. For clearance, a very high confidence in finding no residual contamination is required, which means that there should be a low probability of finding any positive samples. This, in turn, leads to a very large number of samples being required.

Our current approach to the statistical design of clearance sampling is to use acceptance sampling. This is a branch of statistics used in quality control to limit defective units. It deals with the question of: “What can I say about the entire lot, based on sampling a few items from that lot?” It allows for statements such as: “If every sample so far has been negative, can I say something about chances that any remaining samples will be positive?” Unfortunately, in the limiting case of requiring 95% confidence that no sample would be positive, the entire area of building must be sampled, which does not present any advantage. The current implementation of acceptance sampling, however, assumes that all samples are independent of each other. But we know that physical processes of decontamination are spatially correlated, and we next plan to implement ways to use that knowledge to reduce the effective number of samples necessary to achieve the same levels of confidence as under the independent assumption.

Reference

E.H. Isaaks and R.M. Srivastava (1989). *An Introduction to Applied Geostatistics*, Oxford University Press, Oxford, p. 278.

EPA (1997). *Multi-agency Radiation Survey and Site Investigation Manual (MARSSIM)*. Washington, DC, U.S. EPA, DOE, NRC, and DOD: NUREG-1575, EPA 402-R-97-016, <http://www.epa.gov/radiation/marssim/index.html>

EPA. (2000). *Guidance for the Data Quality Objectives Process, EPA QA/G-4. Final Report*, EPA/600/R-96/055, U.S. Environmental Protection Agency, Washington, DC, August 2000. <http://www.epa.gov/radiation/marssim/index.html>

6 Surface Sample Collection Efficiency

Gary S Brown¹, Rita G Betty¹, John E Brockmann¹, Daniel A Lucero¹, Caroline A Souza¹, Kathryn S Walsh¹, Raymond M Boucher², Mathew Tezak³, Mollye C Wilson³, Todd Rudolph⁴, H D Alan Lindquist⁵, and Kenneth F Martinez⁶*

6.1 Overview

Several methods are used in collecting samples to determine the degree of contamination of a building or other area of interest. For hard, indoor areas, swabs, wipes, and vacuum methods, shown in Figure 6-1, are commonly used. Effective sample collection and extraction are essential for reliable characterization and restoration of any site contaminated by a biological agent release. Analyzed samples provide information on initial agent concentration and location, and confirmation that the clean-up goal is achieved.

However, the efficiencies and limits of detection for recommended sample collection methods have never been rigorously determined for aerosol deposited biological agents. A recent GAO report (Government Accounting Office 2005) listed as important issues: “How efficient are the various testing methods, and what minimum amounts of anthrax spores have to be present if anthrax is to be detected by these methods?” and “How effective are the various methods for extracting material from samples for analysis?” As a result of this uncertainty, the interpretation of analytical results may be misleading or even incorrect, and the consequence of an incorrect clean-up verification analysis has the potential for catastrophic economic and social impact.



Figure 6-1. Swabs, wipes and vacuum filter socks used for sampling.

*¹Sandia National Laboratories, Albuquerque, NM; ²Orion International Technologies, Albuquerque, NM;

³American Staff Augmentation Providers, Albuquerque, NM; ⁴Tactical Staffing Resources, Albuquerque, NM;

⁵United States Environmental Protection Agency Homeland Security Research Center, Cincinnati, OH; ⁶National Institute of Occupational Safety and Health, Cincinnati, OH

To address this issue, SNL carried out extensive tests in an aerosol test chamber (see Figure 6-2) and developed rigorous experimental protocols that enable precise sampling efficiency measurements. Collection method efficiencies and limits of detection for swab, wipe, and vacuum surface sample collection methods for both porous and non-porous surfaces seeded by aerosol deposition of powdered *Bacillus atrophaeus* spores were determined. Collection from the non-porous surfaces, stainless steel and painted wallboard, was accomplished using polyester swabs and polyester/rayon blend wipes wetted with de-ionized water, and vacuum filter sock methods. Collection from the porous surfaces, bare concrete and carpet, was accomplished using the vacuum filter sock method. Details of this work are presented in Brown, *et al.*, 2006a,b,c.



Figure 6-2. Photos of aerosol test chamber and array of samples laid out for exposure.

6.2 Results

The experimental results are presented in terms of extraction efficiencies, recovery efficiencies, and collection efficiencies, where

$$(\text{collection } \eta) \times (\text{extraction } \eta) = (\text{recovery } \eta).$$

These parts of the sampling process are illustrated in Figure 6-3.



Figure 6-3. Photos illustrating collection, extraction and recovery, left to right.

Measured extraction efficiencies and recovery efficiencies, along with the calculated collection efficiencies, are presented in Table 6-1 for swabs on stainless steel and painted wallboard. Table 6-2 shows similar data for wipes on stainless steel and painted wallboard, Table 6-3 for vacuum filter socks on stainless steel and painted wallboard, and Table 6-4 for vacuum filter socks on

carpet and bare concrete surfaces. The first thing to note is that the overall recovery efficiencies in these tables are not particularly high, ranging from a high of ~40% for swabs on stainless steel or painted wallboard surfaces, to a low of ~17% for the vacuum on bare concrete. These numbers are substantially lower than unity, which suggests that a given reading obtained by analyzing a surface sample actually corresponds to a significantly higher contamination level in the region of interest.

Table 6-1. Extraction and recovery efficiencies (η) for the swab method with polyester medium from stainless steel and painted wallboard surfaces

Statistic	Swab Extraction η (n = 60)	Stainless Steel		Painted Wallboard	
		Recovery η (n = 40)	Collection η (n = 40)	Recovery η (n = 40)	Collection η (n = 40)
Mean	0.760	0.414	0.544	0.405	0.533
Median	0.762	0.395	0.520	0.375	0.494
Standard Error (\pm)	0.055	0.027	0.017	0.035	0.030
Variance	0.017	0.028	0.011	0.054	0.037

Table 6-2. Extraction and recovery efficiencies (η) for the wipe method with polyester/rayon medium from stainless steel and painted wallboard surfaces

Statistic	Wipe Extraction η (n = 60)	Stainless Steel		Painted Wallboard	
		Recovery η (n = 40)	Collection η (n = 40)	Recovery η (n = 40)	Collection η (n = 40)
Mean	0.514	0.346	0.674	0.285	0.555
Median	0.514	0.311	0.605	0.261	0.507
Standard Error (\pm)	0.011	0.019	0.014	0.024	0.020
Variance	0.007	0.015	0.008	0.023	0.016

Table 6-3. Extraction and recovery efficiencies (η) for the vacuum method with filter sock medium from stainless steel and painted wallboard surfaces

Statistic	Sock Extraction η (n = 60)	Stainless Steel		Painted Wallboard	
		Recovery η (n = 40)	Collection η (n = 40)	Recovery η (n = 40)	Collection η (n = 40)
Mean	0.677	0.289	0.426	0.248	0.366
Median	0.689	0.264	0.391	0.227	0.336
Standard Error (\pm)	0.016	0.023	0.013	0.024	0.015
Variance	0.013	0.019	0.006	0.021	0.008

Table 6-4. Extraction and recovery efficiencies (η) for the vacuum method with filter sock medium from carpet and bare concrete surfaces

Statistic	Sock Extraction η (n = 60)	Carpet		Bare Concrete	
		Recovery η (n = 40)	Collection η (n = 40)	Recovery η (n = 44)	Collection η (n = 44)
Mean	0.677	0.282	0.417	0.189	0.279
Median	0.689	0.259	0.382	0.160	0.236
Standard Error (\pm)	0.016	0.021	0.011	0.021	0.013
Variance	0.013	0.018	0.005	0.020	0.007

Comparing across sampling methods, the data in the first three tables for stainless steel and wallboard surfaces indicate that the process of extracting spores from the sampling medium is most efficient for swabs (77%), followed by vacuum socks (68%), and least efficient for wipes (51%). However, the collection efficiency for the wipes is somewhat higher than for the other two methods, which partially compensates for the lower extraction efficiency. For the recovery efficiencies, the swabs are the most efficient at ~40%, with the wipes and vacuum socks are comparable but somewhat lower at ~ 30-35% efficiencies.

Comparing across surface materials, the collection efficiencies for painted wallboard are generally close to, or slightly lower than, those for stainless steel. Vacuum samples were taken for a wider range of materials, however, and those data show that the collection efficiency, and thus the overall recovery efficiency, is significantly lower for concrete than the other materials.

The experimentally determined recovery efficiencies can be converted into limits of detection for the different sample collection methods.

Table 6-5 gives those results for characterization samples, where quantitative results for spore levels would be required.

Table 6-6 gives similar results for characterization samples, where only qualitative results (presence/absence) would be required. This, in turn, places less stringent requirements on the measurements. The conversion from “recovery efficiencies” to “detection limits” accounts for the fact that the different collection methods cover substantially different sized sample areas. Thus, although the vacuum methods have the worst recovery efficiencies, this collection method also has the best limits of detection.

Table 6-5. Derived detection limits for characterization samples, where quantitative results are required.

Limit of Detection	Collection Method		
	swab	wipe	vacuum
CFU/sample area	100-150	400-600	400-600
CFU/cm ²	4.0-6.0	0.4-0.6	0.04-0.06
CFU/m ²	40000-60000	4000-6000	400-600

Table 6-6. Derived detection limits for clearance samples, where only qualitative results are required.

Limit of Detection	Collection Method		
	swab	wipe	vacuum
CFU/sample area	10-15	15-20	15-20
CFU/cm ²	0.4-0.6	0.015-0.02	0.0015-0.002
CFU/m ²	4000-6000	150-200	15-20

The recovery efficiencies determined in these experiments can be input into the BROOM tool. This would produce contamination maps that are properly corrected for the recovery efficiency of the sample collection method, as well as allowing samples obtained using different collection methods to be correctly compared with each other.

References

Brown, G.S., R.G. Betty, et al. 2006a. *Bacillus atrophaeus* Spore Recovery and Collection Efficiency for Non-Porous Surfaces Using Polyester Swabs. Manuscript submitted to *Journal of Applied Microbiology*, May 2006.

Brown, G.S., R.G. Betty, et al. 2006b. *Bacillus atrophaeus* Spore Recovery and Collection Efficiency for Non-Porous Surfaces Using Polyester/Rayon Blend Wipes. Manuscript submitted to *Applied and Environmental Microbiology*, May 2006.

Brown, G.S., R.G. Betty, et al. 2006c. *Bacillus atrophaeus* Spore Recovery and Collection Efficiency for Porous and Non-Porous Surfaces Using a Vacuum Filter Sock. Manuscript submitted to *Environmental Science & Technology*, May 2006.

Government Accounting Office 2005. Report to the Chairman, Subcommittee on National Security, Emerging Threats, and International Relations, House Committee on Government Reform, House of Representatives. *Anthrax Detection Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results*. March 2005. GAO-05-251. pg 76.

7 Evaluation of Spore Viability Analysis by Flow Cytometry

Gary S Brown¹, Caroline A Souza¹, Kathryn S Walsh¹, Raymond M Boucher², Mathew Tezak³, Mollye C Wilson³*

7.1 Overview

Restoring a transportation facility requires the analysis of a large number of samples, both environmental samples and biological indicators, during the characterization, remediation verification, and clearance stages (Corrigan 2003; Teshale, et al 2002). Analysis of these samples is currently accomplished by traditional culture based methods which determine spore viability by visual inspection for growth in a culture broth 7 to 14 days after inoculation. This analytical method introduces a significant delay to the restoration process and is a limiting factor in the restoration schedule. A rapid assay to determine spore viability would reduce the time for restoration and consequently reduce the economic impact at local and national levels.

For a contamination event involving a *Bacillus anthracis* spore release, remediation is quite likely to be done using fumigation with chlorine dioxide. In this case, biological indicators with spores of a different *Bacillus* species would be placed in the building prior to fumigation, then analyzed for growth after fumigation concentration and time parameters are met.

With the goal of developing a rapid assay to assess viability of biological indicator organisms, and thus fumigation effectiveness, we are developing a method to use flow cytometric detection of rapidly germinated and thus viable *Bacillus atrophaeus* cells from fumigated spore strips. This approach is based on the fact that only viable spores (spores capable of germination and reproduction) will germinate, and that intercalating nucleic acid dyes will stain germinated cells, but not spores. The DNA stained cells would then be detectable by flow cytometry. One advantage of this approach is that instrumentation to carry out these measurements is readily available throughout the national public health laboratory network.

This method involves development of several steps including: 1) a method for rapid and complete spore germination, 2) characterization of *Bacillus atrophaeus* biological indicator spore viability under chlorine dioxide fumigation at lethal and sub-lethal doses, and 3) differentiation and enumeration of germinated cells from fumigated population using flow cytometer with nucleic acid staining. More details of this work are in Brown, et al. 2006.

7.2 Results and Discussion

The first step, spore germination rate enhancement was done using an optimized ammonium chloride chemical germination activator with amino acid initiators. Data are presented in Figure 7-1, where “aa” indicates amino acids addition, “NH₄Cl” indicates ammonium chloride addition, and “D-cyclo” indicates D-cycloserine addition. As indicated, ammonium chloride activation

* ¹Sandia National Laboratories, Albuquerque, NM; ²Orion International Technologies, Albuquerque, NM;

³American Staff Augmentation Providers, Albuquerque, NM

and D-cycloserine auto-inhibition mitigation greatly improves germination rates over amino acids alone, and ensures complete germination of all viable spores in 2 hours.

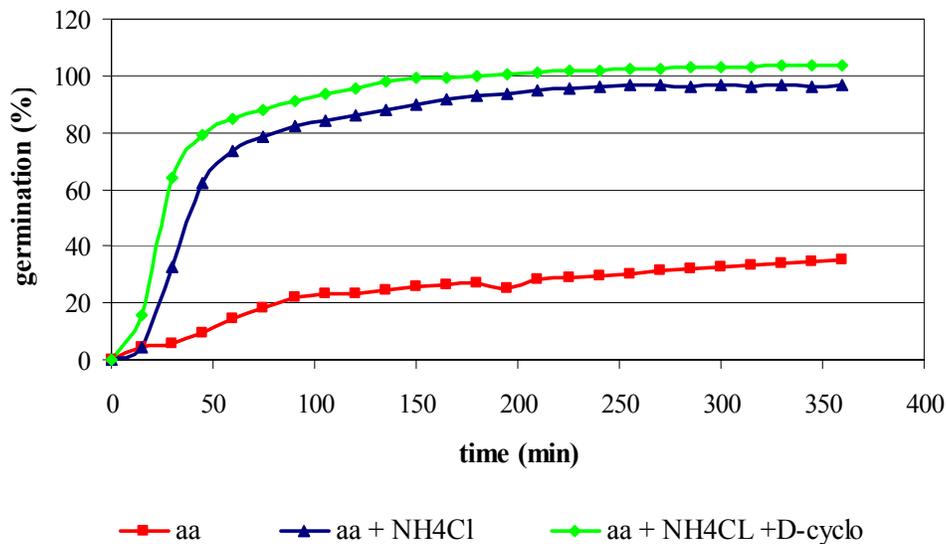


Figure 7-1. Germination rates for selected enhancement solutions.

The second step, investigation of chlorine dioxide sterilization properties at 750 ppm (2.1 mg/l), 25°C, and 75% relative humidity was conducted using standard *Bacillus atrophaeus* spore disks contained in a Tyvek package at nominally 6 log, 5 log, or 4 log spores per disk. Exposure times of 30, 90, 180, and 300 minutes were estimated from 2,500, 5,000, and 10,000 ppm data published by Jeng and Woodworth (1990) to achieve 2 log, 4 log, 5 log, and 6 log reduction, respectively, in viable spore count at 750 ppm.

Following exposure for the specified time period, residual chlorine dioxide, absorbed by the Tyvek package, was neutralized by placing the spore disk package into 0.02% sodium thiosulfate for 15 seconds. Following exposure and neutralization, spores were removed from the disk surface by sonicating in an ultrasonic bath. The spore suspension was then heat treated at 65°C for 60 minutes to kill any bacterial vegetative cells and fungal spores which may be present in the suspension. Five log serial dilutions (10^{-1} to 10^{-5}) of the extracted spore suspension were prepared in sterile de-ionized water and spread to Petrifilm Aerobic Plate Count Media for enumeration.

Comparison of the observed and estimated log reduction values, using the Wilcoxon Matched-Pairs Signed-Ranks Test, indicates no significant difference in log reduction values at a 0.05 significance level ($p \leq 0.5625$), validating the mathematical model derived from the Jeng and Woodworth (1990) data. Observed and estimated values are presented in Table 7-1. Log reduction is non-linear because of spore survivor tailing and is noted in both data sets (Figure 7-2). Spore survivor tailing should be expected in any fumigation where the biological indicator disks are not pre-humidified.

Table 7-1. Estimated and observed spore log reduction times

Log Concentration (CFU)	Estimated Exposure Time (min)	Observed Exposure Time (min)
6	0	0
5	10	15
4	31	35
3	46	60
2	82	100
1	180	165
0	300	295

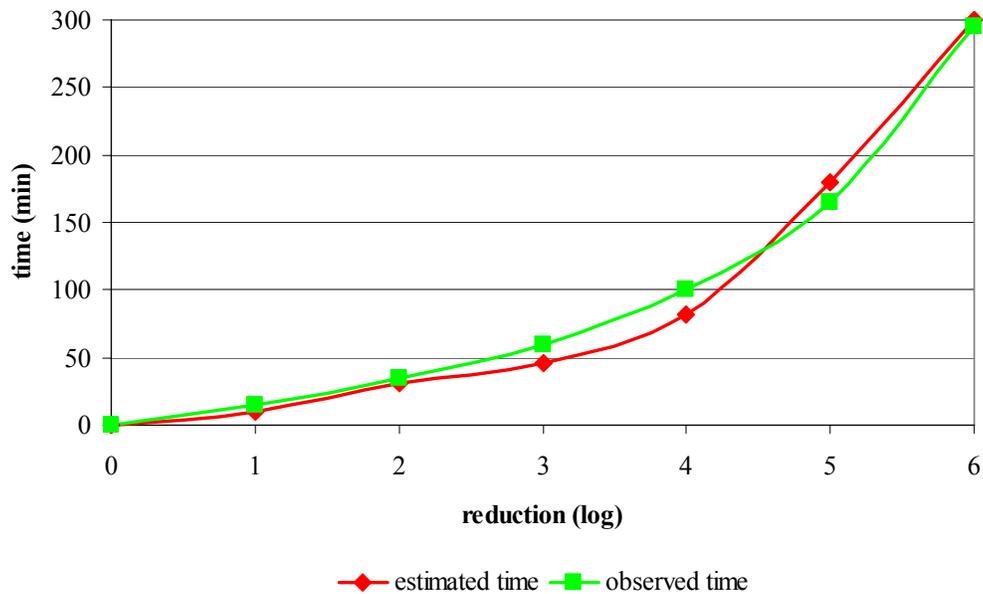


Figure 7-2. Reduction in viable spores with time of exposure to chlorine dioxide: Red diamonds and curve: estimated values based on literature data. Green squares and curve: observed values.

To determine the effect of the neutralization procedure on outcome, a number of biological indicator disks were fumigated for the specified times under the same test conditions, but were not subjected to the neutralization procedure. This was done to determine the residual chlorine dioxide effect on observed log reduction times. Without the neutralizer, the fumigant can continue to react with the spores during sample processing, leading to further decrease in spore viability. The observed 6 log reduction time with neutralization is 120 minutes longer than the observed 6 log reduction time without neutralization (Figure 7-3). Thus reported log reduction times obtained without use of a neutralizer may significantly underestimate actual times required for a 6 log reduction.

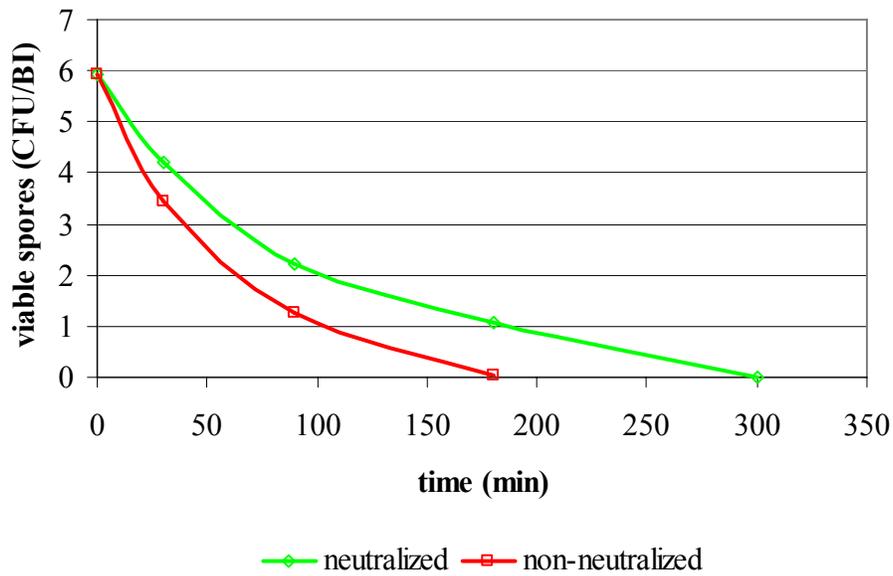


Figure 7-3. Reduction in viable spores with time of exposure to chlorine dioxide: experimental values with and without use of neutralizer.

To determine the effect of initial spore concentration on reduction times, biological indicator disks seeded at nominal 6 log, 5 log, and 4 log spore concentrations were fumigated for the specified times under the same test conditions. The observed time to complete sterilization was dependent on initial concentration. However, while the time required for a one log reduction (D value) was the same for all initial concentrations at approximately 15 minutes, the rate of reduction to complete sterilization was non-linear (Figure 7-4), because of the spore survivor tailing as noted previously. Thus, for complete sterilization without pre-humidification, a 4 log initial concentration requires 100 minutes rather than 60, a 5 log initial concentration requires 180 minutes rather than 75, and a 6 log initial concentration requires 300 minutes rather than 90.

The third step in this work is the differentiation and detection of the vegetative cells using flow cytometry. Figure 7-5 shows a schematic of the experimental apparatus. Flow cytometry can differentiate and enumerate cell types by either nucleic acid stained fluorescence or light scattering due to morphological differences.

Nucleic acid stained fluorescence was selected as the flow cytometry differentiation method. A proprietary fluorescent dye, Syto 9, which is excited at 490 nm and emits in the green at 532 nm and selectively stains nucleic acid in vegetative cells was used. Figure 7-6 shows a photomicrograph of *Bacillus atrophaeus* vegetative cells stained with the Syto 9 dye and excited at 490 nm.

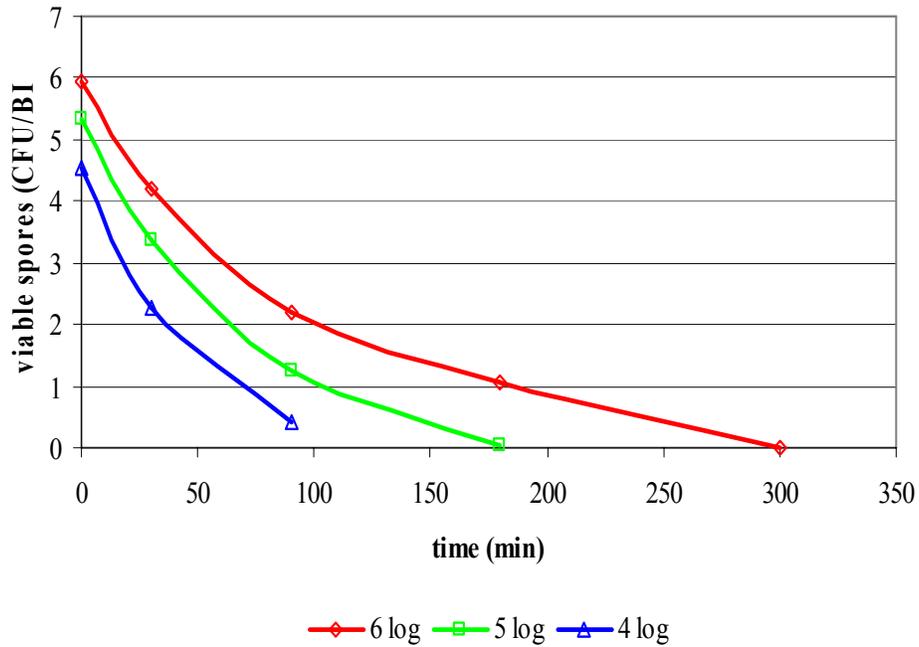


Figure 7-4. Reduction in viable spores with time of exposure to chlorine dioxide for BIs with three different spore concentrations.

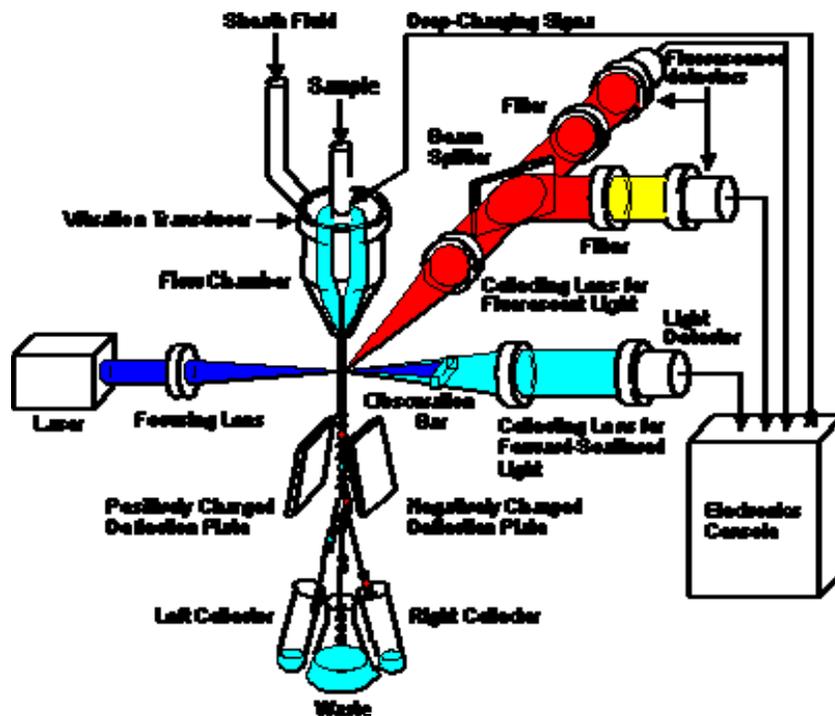


Figure 7-5. Schematic of flow cytometer used for detection of vegetative cells.

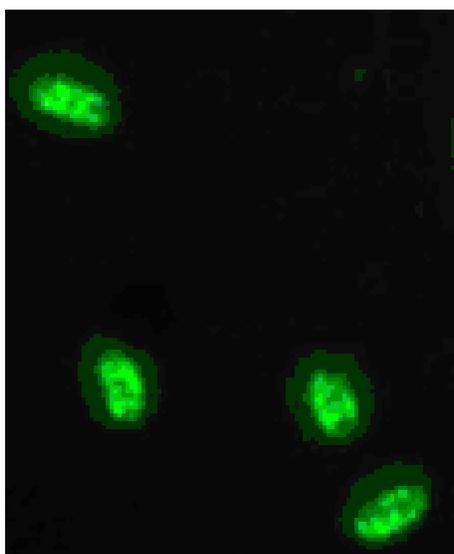


Figure 7-6. *Bacillus atrophaeus* cells stained with Syto 9 fluorescent dye.

Figure 7-7. shows a comparison between flow cytometry results and culture analysis of chlorine dioxide treated *Bacillus atrophaeus*. For short fumigation exposures producing populations with high numbers of survivors, the two methods agree within the experimental uncertainty. However, for longer fumigation exposures with fewer survivors in the population, there is significant disagreement.

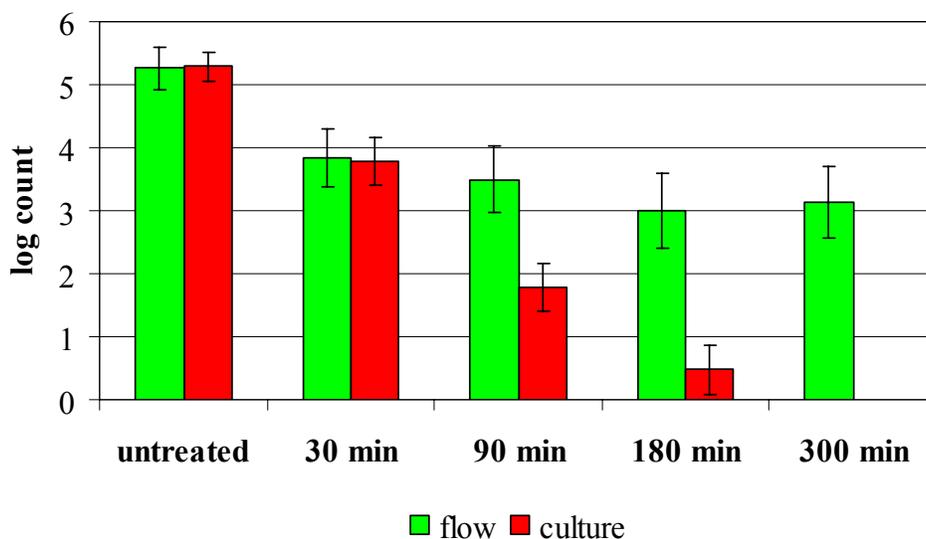


Figure 7-7. Comparison between flow cytometry and culture results.

The disagreement suggests the sterilization mechanism of chlorine dioxide does not disrupt the spore coat and prevent germination by elimination of germination-inducing receptor sites. A comparison by SEM of untreated spore material and spore material treated by 750 ppm chlorine dioxide for 30 minutes (2 log reduction) was conducted to examine the premise that chlorine dioxide exposure produces spore coat disruption. Results of the SEM micrograph comparison show no spore coat disruption of untreated spores and approximately 50% disruption or lysing of spores in the treated material (Figure 7-8). A 30 minute treatment at 750 ppm chlorine dioxide produces a 2 log reduction in viable spores or 99% (verified by culture), so only 1% of the spores should be intact after 30 minutes if the sterilization mechanism is spore coat disruption. A population with 50% spore coat disruption, but 99% non-viability (incapable of outgrowth and reproduction) suggests a sterilization mechanism that allows spore germination, but prevents cell outgrowth and reproduction.

To evaluate this idea, spore material exposed to chlorine dioxide for 300 minutes with no demonstrated growth on culture (0% viable spores), was extracted, stained with Syto 9, and examined with an epi-fluorescence microscope. Germinated and stained cells were observed (Figure 7-9). The germinated but non-viable cells represent approximately 0.1% of the population, and the limit of detection for the flow cytometry viability assay utilized in this study for a chlorine dioxide fumigated population.

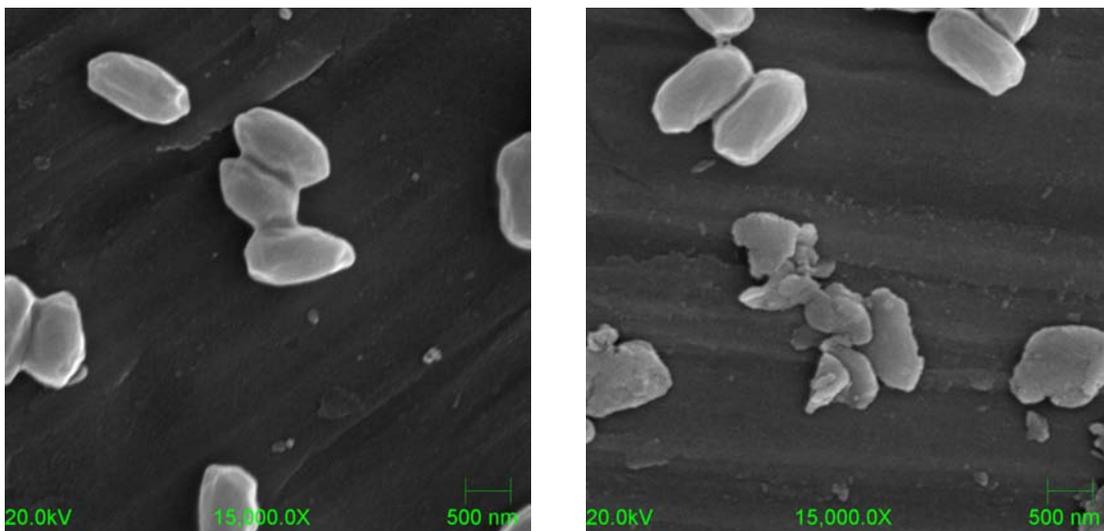


Figure 7-8. Untreated *Bacillus atrophaeus* spores (left) and 30 minute chlorine dioxide treated *Bacillus atrophaeus* spores (right).

The limit of detection may be improved through the use of differential staining. While all vegetative cell nucleic acids are stained with Syto 9, only cells with impaired cell wall integrity (non-viable) are stained by propidium iodide. Propidium iodide is excited at 490 nm as is Syto 9, but emits at red wavelengths rather than green. The difference in emission wavelength can be differentiated and only Syto 9 stained viable cells enumerated by flow cytometry and the non-viable, but germinated, population (propidium iodide stained) deleted (Figure 7-10).

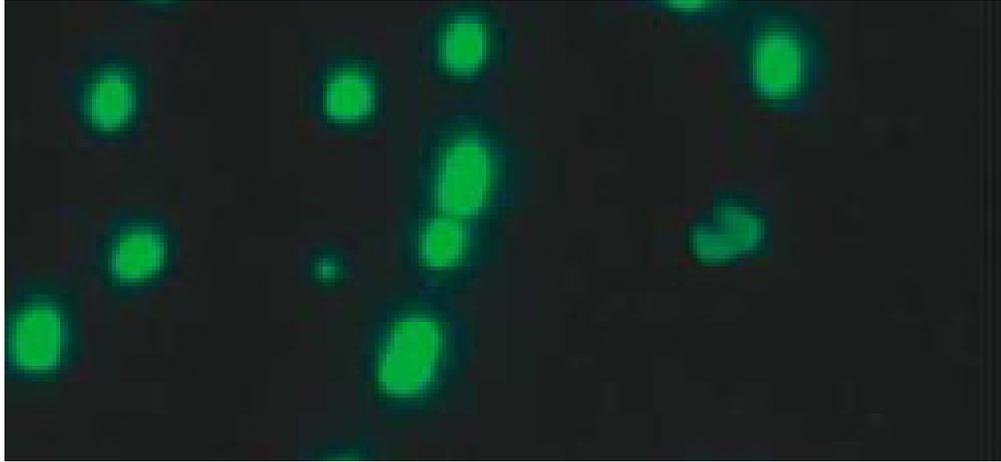


Figure 7-9. Syto 9 stained *Bacillus atrophaeus* cells after 5 hour fumigation

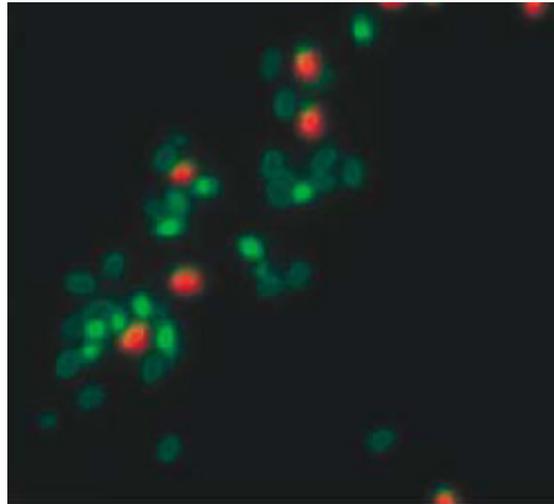


Figure 7-10. *Bacillus atrophaeus* germinated cells stained with Syto 9 (green, viable) and propidium iodide (red, non-viable).

This work presents data indicating that the mechanism of action for sterilization of *Bacillus atrophaeus* spores by chlorine dioxide fumigation does not produce spores with disrupted or lysed spore coats. It is possible for germination to occur from non-viable spores, as well as compromise of the spore coat. The fluorescent stain Syto 9 appears to be selective for all germinated cells, but is non-selective for non-viable germinated cells. Alternative stains thus need to be further investigated.

Flow cytometry detection promises to be rapid, providing results on the minute timescale with high throughput, on the order of 500 samples per day. However, the sensitivity is still problematic for chlorine dioxide fumigation at 0.1% of the total spore concentration. Improvements in sensitivity using differential fluorescent staining are possible.

References

- Brown, G.S., C.A. Souza, K.S. Walsh, R.M. Boucher, M.S. Tezak, and M.C. Wilson. 2006. *Low concentration chlorine dioxide sterilization of Bacillus atrophaeus spores*. Manuscript in preparation.
- Corrigan, G.M. 2003. Spore hunt. *Military Medical Technology Online Archives*. Volume 7 Issue 6. Accessed May 25, 2006. Available from : URL: <http://www.military-medical-technology.com/article.cfm?DocID=252>
- Jeng, D.K. and A.G. Woodworth. 1990. Chlorine dioxide gas sterilization under square-wave conditions. *Applied and Environmental Microbiology*. 56(2):514-519.
- Teshale E.H., J. Painter, G.A. Burr, P. Mead, S.V. Wright, L.F. Cseh, *et al.* 2002. Environmental sampling for spores of *Bacillus anthracis*. *Emerging Infectious Disease*. Volume 18 Issue 10. Accessed May 22, 2006. Available from: URL: <http://www.cdc.gov/ncidod/EID/vol18no10/02-0398.htm>

8 Summary and Conclusions

The events of Fall 2001 following the circulation of letters containing *Bacillus anthracis* spores have shown that the restoration of a large building after contamination with a bioagent is a large, complex operation. The Bio Restoration DDAP, a joint project between LLNL and SNL funded by DHS, is designed to facilitate the process of restoring a major transportation facility by taking a systems approach to the restoration process. The DDAP included tasks that addresses the needs of each stage of restoration: 1) Development of the Remedial Action Plan/Characterization, 2) Remediation, 3) Remediation Verification, 4) Clearance, and 5) Refurbishment. This report contains references to much of the work of the DDAP, but is primarily focused on the technology development done at SNL.

The Building Restoration Operations Optimization Model (BROOM) is a software product developed to assist in the restoration of major transport facilities in the event of an attack involving chemical or biological materials. It will greatly assist the sampling process by automating the error-prone process of manually recording sample position information and analysis results, as well as providing data-management and rapid visualization capabilities. It was demonstrated at a joint Sandia/NIOSH exercise was conducted in Albuquerque, NM from February 16–25, 2005 at a closed SNL facility. This exercise involved the release of a fluorescent powder as a simulant for a bioagent, and provided an extensive dataset on aerosol dispersion in a building as well as feedback on the BROOM system from potential users at NIOSH. The BROOM system was also exhibited during the final DDAP demonstration at SFO on January 25-26, 2006 for representatives from DHS and a variety of airports and transportation facilities.

Many stages of restoring a building involve taking, and analyzing environmental samples. Analyzed samples provide information on initial agent concentration and location, and confirmation that the clean-up goal is achieved. Extensive tests in an aerosol test chamber using rigorous experimental protocols enabled precise sampling efficiency measurements to be done at SNL for aerosol deposited biological agents on both porous and non-porous surfaces. The overall recovery efficiencies for powdered *Bacillus atrophaeus* spores are not particularly high, ranging from a high of ~40% for swabs on stainless steel or painted wallboard surfaces, to a low of ~17% for the vacuum on bare concrete. These numbers are substantially lower than unity, which suggests that a given reading obtained by analyzing a surface sample actually corresponds to a significantly higher contamination level in the region of interest. The experimentally determined recovery efficiencies can be converted into limits of detection, which accounts for the fact that the different collection methods cover substantially different sized sample areas. This conversion that showed that, although the vacuum methods have the worst recovery efficiencies, this collection method also has the best limits of detection. These results are being used by CDC and EPA in revising their recommended sampling protocols.

Analyzing samples for the presence of a bioagent is likely to represent a significant part of the restoration schedule. Analysis is currently accomplished by traditional culture based methods which can take 7 to 14 days. A rapid assay to determine spore viability would reduce the time for restoration and consequently reduce the economic impact at local and national levels. SNL investigated the use of developing a method to use flow cytometric detection of rapidly germinated and thus viable *Bacillus atrophaeus* cells from fumigated spore strips. This work

indicates that the mechanism of action for fumigation of *Bacillus atrophaeus* with chlorine dioxide does not result in the lysing of most of the spores. The fluorescent stain Syto 9 appears to be selective for all germinated cells over undamaged spores, but is non-selective for non-viable germinated cells. Flow cytometry detection promises to be rapid, providing results on the minute timescale, and have high throughput, on the order of 500 samples per day. The Syto 9 dye used in this work had insufficient selectivity and sensitivity, so we are investigating the potential to improve the method by using alternate stains.

Distribution

E-copies

5	Dawn Myscofski U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Jon Herrmann USEPA Facilities 26 West Martin Luther King Drive Mail Code: 163 Cincinnati, OH 45268
5	Theresa Lustig U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Alan Lindquist USEPA Facilities 26 West Martin Luther King Drive Mail Code: 163 Cincinnati, OH 45268
3	Lance Brooks U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Deborah McKean USEPA Facilities 26 West Martin Luther King Drive Mail Code: 271 Cincinnati, OH 45268
1	Beth George U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Cindy Sonich-Mullin USEPA Facilities 26 West Martin Luther King Drive Mail Code: 163 Cincinnati, OH 45268
1	John Vitko U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Oba Vincent USEPA Facilities 26 West Martin Luther King Drive Mail Code: 163 Cincinnati, OH 45268
1	Jeff Stiefel U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Daniel Hawthorne USEPA REGION 8 999 18th Street Suite 300 Mail Code: 8OIG Denver, CO 80202-2466
1	Caroline Purdy U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Eric Koglin USEPA National Homeland Security Research Center 944 East Harmon Avenue Las Vegas, NV 89119
1	Mike McLachlin U.S. Dept. of Homeland Security Washington, D.C. 20528	1	Dennisses Valdes USEPA Env. Response Team - West 4220 South Maryland Parkway Building D, Suite 800 Las Vegas, NV 89119
1	Julius Chang U.S. Dept. of Homeland Security Washington, D.C. 20528		
1	Rick Turville U.S. Dept. of Homeland Security Washington, D.C. 20528		
1	Andrew Avel USEPA Facilities 26 West Martin Luther King Drive Mail Code: 163 Cincinnati, OH 45268		

Distribution (continued)

1	Nancy Adams USEPA Mailroom Mail Code: E343-06 Research Triangle Park, NC 27711	1	Daniel Powell USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 5203P Washington, DC 20460
1	Paul Lemieux USEPA Mailroom Mail Code: E305-01 Research Triangle Park, NC 27711	1	Elizabeth Southerland USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 5204G Washington, DC 20460
1	Blair Martin USEPA Mailroom Mail Code: E343-04 Research Triangle Park, NC 27711	1	David Wright USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 2491T Washington, DC 20460
1	Joseph Wood USEPA Mailroom Mail Code: E343-06 Research Triangle Park, NC 27711	1	Max Kiefer NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226
1	Peter Jutro USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 8801R Washington, DC 20460	5	Ken Martinez NIOSH 4676 Columbia Parkway, MS-R11 Cincinnati, OH 45226
1	Carlton "Jeff" Kempter USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 7510P Washington, DC 20460	1	Rob McCleery NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226-1998
1	Walter Kovalick USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 5203P Washington, DC 20460	1	Greg Burr NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226
1	Mark Mjones USEPA Headquarters Ariel Rios Building 1200 Pennsylvania Avenue, N. W. Mail Code: 5104A Washington, DC 20460	1	Brad King NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226
		1	Chad Dowell NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226

Distribution (continued)

1	Donnie Boomer NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226	1	MS 0384	Art Ratzel, 1500
		1	MS 0701	John Merson, 6110
		1	MS 0734	J. Bruce Kelley, 6215
1	Kevin Dunn NIOSH 4676 Columbia Parkway, Cincinnati, OH 45226	1	MS 0734	Pauline Ho, 6215
		5	MS 0734	Mark Tucker, 6215
		1	MS 0734	Wayne Einfeld, 6215
		1	MS 0734	Gary Brown, 6215
		1	MS 0734	Bob Knowlton, 6215
1	James Bennett NIOSH, MS R5 4676 Columbia Parkway Cincinnati, OH 45226	1	MS 0734	Mollye Wilson, 6215
		1	MS 0734	Matt Tezak, 6215
		1	MS 0734	Ray Boucher, 6215
		1	MS 0734	Jonathan Leonard, 6215
1	Stanley Shulman NIOSH, MS R3 4676 Columbia Parkway Cincinnati, OH 45226	1	MS 0734	Caroline Souza, 6215
		1	MS 0741	Rush Robinett, 6210
		1	MS 0741	Marjorie Tatro, 6200
		10	MS 0836	Richard Griffith, 1517
1	Dr. Sushil Sharma US GAO 441 G. St. NW Washington, DC 20548	5	MS 0836	James Ramsey, 1517
		1	MS 0735	Ray Finley, 6115
		1	MS 0735	Sean McKenna, 6115
		1	MS 0735	Chad Peyton, 6115
		1	MS 0824	Wahid Hermina, 1510
1	Ellen Raber Lawrence Livermore National Lab P.O. Box 808, Livermore, CA 94551-0808	1	MS 0836	Patrick Finley, 1517
		1	MS 0836	Brad Melton, 1517
		1	MS 0836	John Brockmann, 1517
		1	MS 0836	Dan Lucero, 1517
1	Don MacQueen Lawrence Livermore National Lab P.O. Box 808, Livermore, CA 94551-0808	1	MS 0836	Todd Rudolph, 1517
		1	MS 1161	Dan Rondeau, 5430
		1	MS 1230	Veronica Lopez, 10531
		1	MS 9004	Jill Hruby, 8100
		1	MS 9004	Pat Falcone, 8110
1	Brent Pulsipher Pacific Northwest National Laboratory PO Box 999, MS-K6-08 Richland, WA 99352	1	MS 9004	Duane Lindner, 8120
		1	MS 9004	Brian Damkroger, 8130
		1	MS 9155	Howard Hirano, 8122

Hardcopies

2	MS 0734	Pauline Ho, 6215
2	MS 9018	Central Technical Files, 8944
2	MS 0899	Technical Library, 4536