

EPA/600/R-17/156 | August 2017 www.epa.gov/homeland-securityresearch

Evaluation of Commercially-Available Equipment for the Decontamination of *Bacillus anthracis* Spores in an Urban Subway System





Office of Research and Development National Homeland Security Research Center This page left intentionally blank

FINAL REPORT

Evaluation of Commercially-Available Equipment for the Decontamination of *Bacillus anthracis* Spores in an Urban Subway System

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

Disclaimer

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's (ORD's) National Homeland Security Research Center (NHSRC), funded, directed and managed this work through Contract Number EP-C-15-002, Task Order 007, with Battelle. This report has been peer and administratively reviewed and has been approved for publication as an EPA document. The views expressed in this report do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

This study was funded through the Underground Transport Restoration Program by the U.S. Department of Homeland Security Science and Technology Directorate under interagency agreement (No. 7095866901).

Questions concerning this document or its application should be addressed to:

Dr. Worth Calfee National Homeland Security Research Center Office of Research and Development U.S. Environmental Protection Agency Mail Code E343-06 Research Triangle Park, NC 27711 919-541-7600

Acknowledgments

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

U.S. Environmental Protection Agency (EPA) Project Team

Worth Calfee (Principal Investigator, EPA, NHSRC) Sang Don Lee (EPA NHSRC) Lukas Oudejans (EPA NHSRC) Shannon Serre (EPA CBRN Consequence Management Advisory Division (CMAD) Leroy Mickelsen (EPA Office of Land and Emergency Management (OLEM) CMAD Mike Nalipinski (EPA OLEM CMAD)

EPA Technical Review Timothy Boe Edward Bazenas

EPA Quality Assurance

Eletha Brady-Roberts Ramona Sherman

EPA Editorial Review

Marti Sinclair

Battelle Memorial Institute

Executive Summary

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

The Underground Transport Restoration (UTR) project is an inter-agency effort. This effort aims to improve the capability for transit systems to quickly and efficiently recover from a biological contamination incident by refining existing methods, tools and protocols for characterization, clean-up, and clearance of contamination in physical structures (i.e., tunnels, stations) and rolling stock (i.e., subway trains). The aim was to evaluate existing sampling, characterization, and decontamination technologies through experimentation, table-top exercises and operational demonstrations to develop guidance and decision frameworks and support tools through interactions of local, state and federal partners.

In this investigation, a survey of commercially-available or fielded equipment was conducted and resulted in three pieces of identified equipment that could be used or rapidly modified for use in dispensing liquid chemicals to decontaminate surfaces following a biological contamination incident. The equipment selected was the MM Sprayers, Air-O-Fan[®] (AOF), and Dust Boss[®] sprayers. This equipment was selected based on rankings provided by a working group comprised of EPA, EPA's technical support contractor for this effort, and stakeholders representing the Transit Authority from across the US. This equipment was subjected to 100 hours of operation with pH-amended bleach (pAB) using smaller proxy equipment designed in consultation with the vendors of the equipment to test for material compatibility with pAB. Based on durability assessment, two pieces of equipment (AOF and Dust Boss sprayers) were further down-selected to participate in a field-scale demonstration at a subway platform/tunnel at Fort A.P. Hill (Bowling Green, VA). For purpose of demonstration, both pieces of equipment were placed atop a flatbed railcar and used to spray water while the railcar was pulled through the subway platform/tunnel at a speed of 1.2 miles per hours (mph). Video and leaf wetness data (5 locations) were collected during this demonstration. The leaf wetness sensor measures the percentage of the capacitive grid that is covered by moisture. Based on the leaf wetness data, review of video, and observer input, a single piece of equipment was selected (AOF sprayer) to perform field scale efficacy tests using *Bacillus atrophaeus (B.g.)* spores as a surrogate for *B*. anthracis (B.a.), the causative organism of anthrax.

Efficacy testing was conducted within Battelle's ambient breeze tunnel (ABT) testing facility. The ABT allowed full-scale implementation as the internal dimensions of this facility were representative of many existing subway tunnels. Decontamination efficacy of operationally sprayed pAB against surrogate *B.g.* was evaluated at target delivery speeds of 1.2 and 2.4 mph, target temperature of 10 degrees Celsius (°C), uncontrolled relative humidity (RH) ranging from 59 to 98 percent (%), vertical and horizontal coupon orientations, and contact times ranging from 30 minutes (min) to 12 hours (overnight) for a total of 4 tests. Ceramic tile resulted $\geq 6 \log$ reduction (LR) at each condition tested (Tests 1-3). Unpainted concrete resulted in LRs ranging

from 1.62 to 2.34 and 1.32 to 3.02 at locations 1 (column) and 2 (floor), respectively. Since concrete was more challenging, Test 4 utilized concrete only with repeat applications (2 and 3) with 30 min contact times between applications. This resulted in increased LR ranging from 3.51 to 4.70 for 2 and 3 applications, respectively. A decontaminant or fumigant technology is considered to be effective if a 6 LR or greater is achieved on the materials tested for a given set of fumigation conditions [sporicidal liquid volume, temperature, and relative humidity (RH)]⁽¹⁾.

Summary of Major Findings

Over the course of the study all testing conducted with ceramic tile resulted in >6 LR of *B.g.*, while no conditions were found that resulted in >6 LR of *B.g* on unpainted concrete. It was observed that neither decontamination delivery speeds of 1.2 or 2.4 mph, nor increased contact times greater than 30 min resulted in a significant effect on LR. Repeat applications of pAB up to 3 each resulted in increased efficacy as shown in Figure ES-1through ES-3.

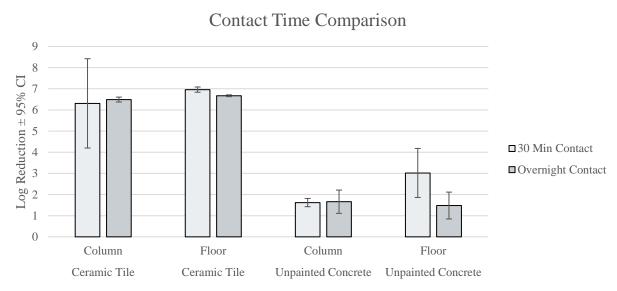


Figure ES-1. 30 min (Test 1) vs overnight contact time (Test 2).

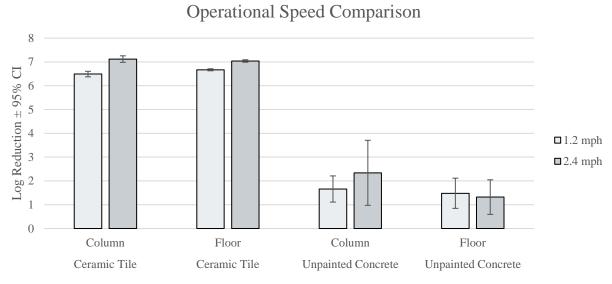


Figure ES-2. 1.2 mph (Test 2) vs 2.4 mph application rate (Test 3)

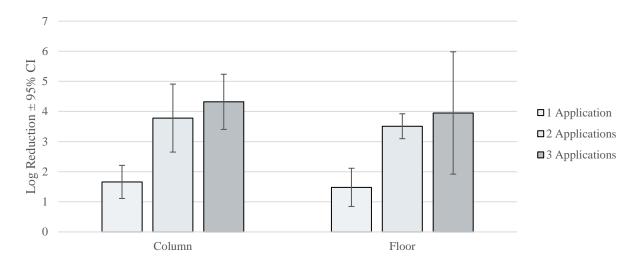


Figure ES-3. Test 2, 4a, and 4b repeat application comparison.

Contents

Ack	now	ledgments iv
Exe	cutiv	ve Summaryve
1.0	Intr	oduction1
2.0	Ma	ket Survey
3.0	Dur	ability Testing
4.0	Fiel	d Scale Demonstration
5.0	Effi	cacy Testing9
	5.1	Test Matrix
	5.2	Biological Organism
	5.3	Test Materials
		Inoculation of Coupons10
	5.5	Spraying Equipment11
	5.6	Ambient Breeze Tunnel and Procedures
	5.7	Coupon Extraction and Biological Agent Quantification14
	5.8	Decontamination Efficacy14
		Surface Damage
6.0		lity Assurance/Quality Control17
	6.1	Equipment Calibration17
	6.2	QC Results
		Operational Parameters17
	6.4	Audits
	6.5	QA/QC Reporting
	6.6	Data Review19
7.0	Res	ults and Discussion
	7.1	Durability Testing
	7.2	Field Scale Demonstration
	7.3	Efficacy Testing
	7.4	Surface Damage to Materials
	7.5	Summary
8.0	Ref	erences

Figures

Figure ES-1. 30 min (Test 1) vs overnight contact time (Test 2).	vi
Figure ES-2. 1.2 mph (Test 2) vs 2.4 mph application rate (Test 3)	vii
Figure ES-3. Test 2, 4a, and 4b repeat application comparison	
Figure 2-2. Air-O-Fan D-40R radial type sprayer.	4
Figure 2-1. MM Sprayers USA MM LG 400 radial fan sprayer.	4
Figure 2-3. Dust Boss DB-30 sprayer.	5
Figure 3-1. Durability test device.	
Figure 4-1. Demonstration equipment on railcar.	7
Figure 4-2. Schematic of Fort A.P. Hill subway tunnel	8
Figure 5-1. Coupon types from left to right: unpainted concrete, ceramic tile on test fixtu	ire.
Orange tab indicates blank coupon.	10
Figure 5-3. Customized AOF Model 2-36 sprayer.	11
Figure 5-2 Liquid inoculation of coupon using a micropipette.	
Figure 5-3. Customized AOF Model 2-36	12
Figure 5-5. AOF and test cart configuration. Figure 5-3. Customized AOF Model 2-36	12
Figure 5-5. AOF sprayer and test cart configuration.	13
Figure 5-4. Ambient breeze tunnel test facility	13
Figure 7-1. MM sprayer failure.	
Figure 7-2. Dust Boss nozzle failure. From left: control, test 1 test 2 and test 3 nozzles	21
Figure 7-3. Durability test flow rate.	21
Figure 7-4. Durability test pressure.	21
Figure 7-5. AOF nozzle failure. From left: control, test 1, test 2, and test 3 nozzles	22
Figure 7-6. AOF sprayer Fort A.P. Hill (FAPH) demonstration % wetness	23
Figure 7-7. Dust Boss sprayer Fort A.P. Hill (FAPH) demonstration % wetness	23
Figure 7-8. 30 min (Test 1) vs overnight contact time (Test 2)	24
Figure 7-9. 1.2 mph (Test 2) vs 2.4 mph application rate (Test 3).	25
Figure 7-10. Test 2, 4a, and 4b comparison	25
Figure 7-11. Test 2, 4a, 4b, and 4c comparison.	26
Figure D1. pH amended bleach (pAB) vs 2% dilute bleach at 2.4 mph	D-2
Figure D2. Efficacy of pH amended bleach (pAB) at varied application speeds	D-3
Figure D3. Mass deposition of water at various delivery speeds.	
Figure D4. Efficacy of multiple applications of pH amended bleach (pAB) and 2% bleach.	D-4

Tables

9
A-1
B-1
B-2

Table B-2. Durability Testing Flow (Continued)	B-2
Table C-1. Commercial Equipment for Subway Decontamination	
Table C-2. Commercial Equipment for Subway Decontamination (Continued)	C-Error!
Bookmark not defined.	
Table D-1. Add-on Inactivation of B. atrophaeus Spores using pH Amended and dilute	2%
Bleach ^a	D-1

List of Appendices

Appendix A Detailed Test Results	A-1
Appendix B Detailed Durability Results	B-1
Appendix C Detailed Market Survey Results	
Appendix D Mod 5 Add-on Test Results	
Appendix E Technology Demonstration Feedback Results	

Abbreviations/Acronyms

AOAC	Association of Analytical Communities
ABT	Ambient Breeze Tunnel
ATCC	American Type Culture Collection
<i>B.a.</i>	Bacillus anthracis
B.g.	Bacillus atrophaeus
BSC	biological safety cabinet
CFU	colony forming units
CI	confidence interval
cm	centimeter(s)
CMAD	Consequence Management Advisory Division
°C	degree(s) Celsius
Decon	decontamination
EPA	U.S. Environmental Protection Agency
FAPH	Fort A.P. Hill
HSRP	Homeland Security Research Program
kg	Kilogram
kPa	kilopascal
Kw	kilowatt
L	liter(s)
lpm	liters per minute
LR	log reduction
μL	microliter(s)
m	meter
mph	miles per hour
mL	milliliter(s)
min	minute(s)
NA	not applicable
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OLEM	Office of Land and Emergency Management
pAB	pH-amended bleach
PBS	phosphate buffered saline
PBST	PBS + 0.1% Triton X-100
PCR	polymerase chain reaction
ppm	parts per million
ppmv	parts per million by volume
psi	pounds per square inch
QA	quality assurance
QAPP	Quality Assurance Project Plan

QC	quality control
QMP	Quality Management Plan
RH	relative humidity
rpm	revolution(s) per minute
S	second(s)
SD	standard deviation
SE	standard error
UTR	underground transportation restoration

1.0 Introduction

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

Contamination of an underground transportation system (i.e., subway tunnel or platform) following a biological terror incident would have a crippling effect on a city's economy and stability. Rapid decontamination following such an incident is paramount for returning to normalcy. To facilitate a rapid mobilization following an incident and to shorten the remediation process, utilization of commercially available equipment is desirable. Equipment that can rapidly be obtained and utilized to dispense liquid sporicides to subway infrastructure would be very useful for underground transportation system decontamination.

In this investigation, a survey of currently fielded equipment or other readily-adaptable commercially available equipment that could dispense liquid sporicides was conducted and compiled into a spreadsheet containing applicable operational specifications. This spreadsheet was evaluated by a working group comprised of EPA, EPA's support contractor for this for effort (Battelle), and transit authority staff. The groups provided ranking scores in one of three categories (Commercial Readiness, Ease of Deployment, and Decontamination ("Decon") Application Rate). The scores across all categories were then aggregated and used to select the top three technologies. A durability assessment was conducted for each piece of equipment to identify potential material compatibility issues with the selected sporicidal liquid, pH-amended bleach (pAB). Due to the large scale of the equipment being investigated, a series of proxy equipment were designed, with input from the equipment manufacturers, using parts from the larger equipment and were tested in triplicate for up to 100 hours of operation. pAB or water (control) flow rate and pressure were recorded at the beginning and end of each day of testing. Any observable changes to operational pressure or flow rate were investigated as potential failure and documented with photographs.

A field demonstration was conducted at Fort A.P. Hill (Bowling Green, VA) facility using the top two performing pieces of equipment as measured by the durability assessment. This facility houses a subway training facility with an approximate 84 meter (m) subway platform and 113 m subway tunnel. Each piece of equipment was operated by spraying water and was pulled through the subway tunnel/platform at a fixed speed of 1.2 miles per hour (mph). Leaf wetness sensors and video were utilized to determine the amount and efficiency of each equipment to deposit liquid onto several complex surfaces and orientations. The leaf wetness sensor measures the percentage of the capacitive grid that is covered by moisture.

Finally, field-scale efficacy tests were conducted to evaluate the performance of the demonstrated equipment for decontamination of materials found in subway tunnels or stations that could become contaminated with biological agents such as *Bacillus anthracis* (*B.a.*) spores. (*B. anthracis* is the bacterial pathogen that causes anthrax.) The efficacy of pAB was evaluated

on both unpainted concrete and ceramic tile contaminated with *Bacillus atrophaeus* (*B.g.*) as a surrogate spore for *B.a.* Decontamination efficacy was determined based on the log reduction (LR) in viable spores recovered from the inoculated samples (with and without exposure to the sporicidal liquids). A decontaminant or fumigant technology is considered to be effective via Association of Analytical Communities (AOAC) (test method 966.04) if a 6 LR or greater is achieved on the materials tested (AOAC material types not used for present study) for a given set of fumigation conditions [sporicidal liquid volume, temperature, and relative humidity (RH)]⁽¹⁾.

The results of this investigation provide decontamination stakeholders and decision makers with high quality, peer-reviewed data on the use of equipment to disperse sporicidal liquids in a subway environment, as a function of the spore type, the material the spore is associated with, temperature, equipment type and sporicidal liquid used.

2.0 Market Survey

A thorough search was conducted for equipment currently fielded or equipment readily capable of disseminating large amounts of liquids in a quick and efficient manner and capable of targeting the multiple surface orientations that exist in a complex subway system. Information such as operational pressure, liquid flow rate, mode of liquid delivery, power requirements, tank size, material of construction, weight, and dimensions were collected and compiled into a spreadsheet that can be found in Appendix C in its entirety. The information was collected using internet search engines, literature review and by leveraging connections within the working group for recommendations based on practical industrial use and knowledge. Members of each part of the working group reviewed the developed spreadsheet and provided ranked scores in one of three categories: Battelle ranked Commercial Readiness, Transit Authorities ranked Ease of Deployment, and EPA ranked Decon Application Rate (Table 2-1). Each category had a possible ranking from 1 to 5, with the highest possible total aggregate score of 15. The summed total of the three categories were aggregated for all groups.

	Score	Brief Description	Long Description
	1	Not at all	not commercially available
	2	Poor	very limited commercial availability
Commercial Readiness	3	Moderate	available, but in limited quantities or select locations
	4	Good	generally available
	5	Excellent	readily available, multiple vendors, any region in US
	1	Not at all	unable to deploy equipment in subway environments
	2	Poor	major technical hurdles to deploy equipment in subways
F C	3	Moderate	some modifications needed for deployment in subways
Ease of Deployment	4	Good	minor modifications or logistical challenges for deployment in subways (i.e., needs flatbed railcar to transport)
	5	Excellent	easily deployed into subways with no modification (i.e., can be directly deployed to subways with no modification or additional equipment required)
	1	Not at all	unable to dispense decontaminants, due to incompatibility or technical issues
	2	Poor	very limited ability to dispense decontaminants, application rate or spray reach is insufficient for subway application
Decon Application Rate	3	Moderate	moderate ability to dispense decontaminants, application rate or spray reach is acceptable for some subway applications
Mutt	4	Good	good ability to dispense decontaminants, application rate or spray reach is acceptable for most/many subway applications
	5	Excellent	superior ability to dispense decontaminants, application rate or spray reach is acceptable for all subway applications

Table 2-1. Equipment Scoring

A total of 22 pieces of equipment were identified from several industrial sectors such as agricultural sprayers, roadway de-icing equipment, construction dust suppression, crowd cooling, firefighting, and insect control, which were divided into six categories (Radial Fan Sprayers, Air Directed Sprayers, Electrostatic Sprayers, Dust Suppression Equipment, Foggers and De-icing Equipment). In some cases, multiple versions or brands of a similar technology existed. To make the final evaluation spreadsheet as concise as possible, one to two representative technologies were selected where market availability and lower cost was preferred. The final raking was achieved by aggregating all scoring across functional working groups per piece of equipment. The top three pieces of equipment were chosen to be carried through for durability testing (Table 2-2). These top three pieces of equipment were all found to be: readily available; able to be deployed in a subway environment with minimal modifications; and capable of dispensing liquid decontaminants into a subway system environment.

Table 2-2. Final Down-Selected Equipment for Subway Decontamination

SCORE	Company	Category	Model #	Web link to sprayer
12.3	MM Sprayers USA	Radial Fan Sprayer	MM LG 400	http://www.mmsprayersusa.com/product/mm-lg- 400-gas-trailer/
11.1	Air-O-fan	Radial Fan Sprayer	D-40R 1,000 Gallon	http://airofan.com/OrchardSprayers/EngineDrive/ D40RModel/D40R1000Gal.aspx
10.8	Dust Boss	Dust Suppression	DB-30	http://www.dustboss.com/products/db-30/

D4 spray tips

Technologies,

(TeeJet

The top scoring piece of equipment was the MM Sprayers USA MM LG 400 (MM Sprayers USA, Lynden, WA) radial fan sprayer (Figure 2-1). This sprayer is typically used in the vineyard or berry-growing industry. It has a 400 liter (L) polyethylene tank, 0.64 m diameter fan, 10 Braglia brass swivel rollover anti-drip nozzle bodies with TeeJet[®] stainless steel hollow cone



Figure 2-2. Air-O-Fan D-40R radial type sprayer.

Figure 2-1. MM Sprayers USA MM LG 400 radial fan sprayer.

Glendale Heights, IL). It uses a A.R. 403 diaphragm pump, powered by a 13 horse power Honda engine. Dimensions of the equipment are 1.2 m width, 1.4 m height, 2.5 m length with a dry weight of 264 kilograms (kg). Typical cost of this unit is approximately \$10,000.

The second ranked piece of equipment was the Air-O-Fan (AOF) D-40R 3800 L (Air-O-Fan Products Corp, Reedley, CA) radial type sprayer (Figure 2-2). This sprayer is typically used in the orchard and nutgrowing industry. Several models of this type of sprayer are available. It has a 3800 L type 304 stainless steel tank and is expandable through use of rapid fill cam lock fittings. The sprayer is powered with 156 John Deere[®] (Deere & Co., Moline, IL) diesel engine and powers a 2-stage 200 pounds per square inch (psi) centrifugal pump and twin steel co-axial fans capable of throwing liquid droplets from 43 m to 82 m. Composite nylon adjustable air vanes house up to two nozzles per vane (total 36). Liquid flow rates are controllable between 1 and 380 liters per minute (lpm). Dimensions of the equipment are 2.7 m width, 1.5-1.8 m height, 6.2 m length and a dry weight of 3838 kg. Equipment costs range from \$60,000 to \$100,000 based on model type and options selected.



Figure 2-3. Dust Boss DB-30 sprayer.

The third ranked piece of equipment was the Dust Boss[®] DB-30 (Figure 2-3) sprayer (BossTek, Peoria, IL). This equipment is used in demolition and other industrial settings as a dust suppression cannon. Several models of this sprayer are available. This sprayer is powered by an electric direct drive motor and is capable of throwing liquid droplets up to 30 m with liquid delivery rates from 5.3 to 10.6 lpm. Standard unit oscillates through a 70 degree pattern and has a manual 0 to 50 degree vertical angle adjustment. Unlike the first two pieces of equipment, this device requires external input for liquid at approximately 50 psi and electric power (60 kilowatt [kw] generator). Equipment costs range from \$22,000 to \$60,000 based on model type but it is worth noting this does not include power generation or pressurized liquid delivery.

3.0 Durability Testing

The ability to use commercially available equipment provides advantages in terms of reduced implementation time, less operators per surface/length, and potential lower overall cost. However, a potential drawback is the equipment may not be fully compatible with many of the sporicidal liquids considered for field-scale remediation. Many of these chemicals are corrosive, such as pAB, and can cause premature material degradation and equipment failure.

To assess this potential in advance of field-scale testing, a laboratory-scale durability test was conducted for the three down-selected pieces of equipment to identify potential material compatibility issues with the selected sporicidal liquid, pAB. Due to the large scale of the equipment being investigated, a series of smaller proxy equipment was designed using parts or representative parts from the larger equipment and were tested in triplicate for up to 100 hours of operation (Figure 3-1). Each vendor was consulted and asked to provide a list of the wetted

components within their larger equipment to aid in design. During testing, pAB (n=3) or water (control, n=1) flow rate and pressure were recorded at the beginning and end of each day using a timed collection into a graduated cylinder and a Wika[®] Model 2135325 National Institute of Standards and Technology (NIST) traceable pressure gauge (Wika Instrument, Lawrenceville, GA). The system was designed such that the liquid exiting the spray nozzles was collected into flexible tubing and recirculated back to the liquid holding tank. Any observable changes were investigated as potential failures and documented with photographs.

During a typical test run, the pAB solution was prepared fresh each day as described in the EPA crisis exemption



Figure 3-1. Durability test device.

requirements for use against B.a. spores⁽²⁾. The solution was prepared by combining one part Germicidal Clorox[®] Bleach (Clorox Corp., Oakland, CA, USA) with eight parts deionized water and one part 5% (v/v) Heinz[®] distilled white vinegar (Kraft Heinz Company, Mendota Heights, MN, USA). The pH was adjusted to 6.5–7.0 with additional vinegar, and the free available chlorine content was measured and acceptable if \geq 8000 parts per million by volume (ppmv). Pressure measurements were collected by briefly starting each sprayer and recording pressure from NIST traceable pressure gauges. Flow rate was measured by a timed collection of fluid exiting each individual spray nozzle. The equipment was then operated for a defined amount of time per test day. After each test, pressure, flow rate, free available chlorine, and pH measurements were collected and recorded.

4.0 Field Scale Demonstration

A field-scale operational demonstration was performed on October 13, 2016 at Fort A.P. Hill (FAPH) in Virginia. The FAPH maintains a subway platform and a tunnel with an approximate 84 m subway platform and 113 m subway tunnel that was used for this testing. Based on performance from the durability testing, the AOF and Dust Boss systems were selected by the EPA working group for use.

Due to weight limitations of the cart intended for use initially (Xinxiang Hundred Percent Electrical and Mechanical Co., Ltd, Model: KPX40T, He'nan Provice, China), a few modifications to the AOF equipment were necessary. At the time of the demonstration, a full-size flatbed railcar became available and was utilized for the demonstration. A smaller D2-36 model was selected and modifications included removal of the pneumatic wheels (unit to be mounted on rail car), exchange of diesel engine for V10 aluminum block gasoline engine, smaller 250-gallon stainless steel tank, and an added 12 foot downward facing spray bar. The AOF and Dust Boss equipment (including 60 kw generator) were placed atop a flatbed rail car (Figure 4-1) and pulled through the subway tunnel and platform at a fixed speed of 1.2 mph using a Maxi railcar mover (Railquip Inc, Atlanta, GA). Each piece of equipment was operated (one at a time) while the railcar was pulled through the entire length of the tunnel. Multiple runs



Figure 4-1. Demonstration equipment on railcar.

were conducted for each piece of equipment.

Leaf wetness sensors were placed at 5 locations within the platform or tunnel sections to assess the distribution of liquid droplets as total percent coverage over a range of orientations as noted in Figure 4-2. In addition, high definition video of each equipment test run was collected from various angles and was provided as a deliverable in addition to the report.

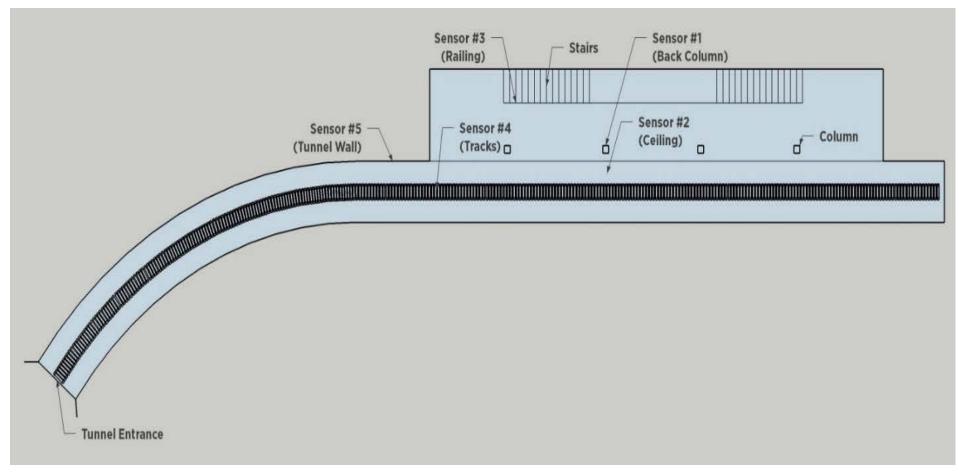


Figure 4-2. Schematic of Fort A.P. Hill subway tunnel.

5.0 Efficacy Testing

This section provides an overview of the procedures used for the field-scale evaluation of commercially-available equipment for spraying sporicidal liquids to inactivate *B.g.* on up to two material types. Testing was performed in accordance with EPA ORD's QA program and Battelle's QA program.

5.1 Test Matrix

The test matrix for the decontamination tests is shown in Table 5-1. Tests 1-3 were performed using two materials commonly found in a subway environment (ceramic tile and unpainted concrete) while Test 4 used unpainted concrete only. Operational parameters were chosen to assess conditions representative of field environments and equipment in the case of a wide area subway contamination event. Testing was conducted at a target of 10 °C, but varied due to the field-scale testing environment.

Table 5-1	pH Amended Bleach Decontamination Test Matrix using the AOF Sprayer at
10 °C	

	Oper	ational Param	eters	
Test Number	Equipment	Contact Time	Number of	Materials
	Speed		Applications	
1	1.2 mph	30 Min	1	Ceramic Tile, Unpainted Concrete
2		Overnight	1	Ceramic Tile, Unpainted Concrete
3		Overnight	1	Ceramic Tile, Unpainted Concrete
4a		Overnight	2*	Unpainted Concrete
4b		Overnight	3*	Unpainted Concrete
4c		Overnight	4*	Unpainted Concrete

*30 min contact time observed between applications.

5.2 Biological Organism

The *B.g.* spores (Lot DJS-BG-004) were supplied in powder form originally obtained from Dugway Proving Ground (Tooele County, UT). The *B.g.* stock spore suspensions were prepared in sterile phosphate-buffered saline (PBS) at an approximate concentration of 1×10^9 colony forming units per milliliter (CFU/mL) and stored at 2 to 8 degrees Celsius (°C). Genomic DNA was extracted from the spores and DNA fingerprinting by polymerase chain reaction (PCR) to confirm the genotype (matches ATCC[®] 9372TM, Manassas, VA). In addition, the number of viable spores was determined by colony count and expressed as CFU/mL. Theoretically, once plated onto bacterial growth media, each viable spore germinates and can yield one CFU although the possibility does exist that multiple spores, if co-located when plating, can also result in one CFU.

5.3 Test Materials

Decontamination efficacy testing was conducted using common subway tunnel materials (ceramic tile and unpainted concrete). Information on these materials is presented in Table 5-2, and a picture of each is presented in Figure 5-1. Material coupons were cut to uniform length and width (Table 5-2) from larger pieces of stock material. Materials were prepared for testing by sterilization via autoclave at 121 °C, 103 kPa for 15 min. Autoclaved coupons were sealed in sterilization pouches (Cat. No. 01-812-50, Fisher, Pittsburgh, PA) to preserve sterility until the

coupons were ready for use. Sterilization was intended to eliminate contamination by endogenous microorganisms.

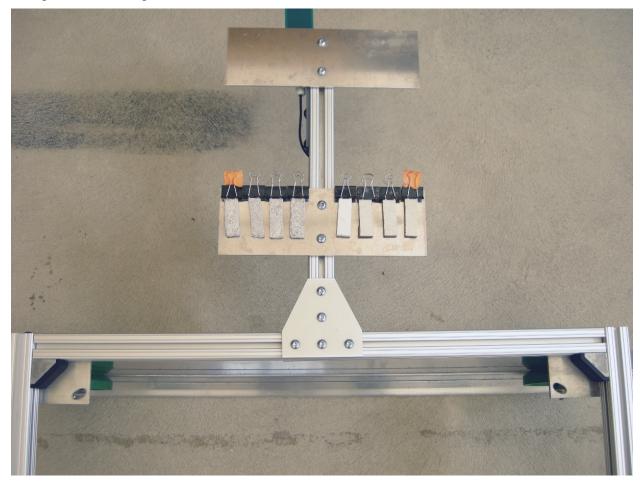


Figure 5-1. Coupon types from left to right: unpainted concrete, ceramic tile on test fixture. Orange tab indicates blank coupon.

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name Location	Approximate Coupon Size, Width x Length x Thickness	Material Preparation
Ceramic Tile	Model: PWHITW91L01	Lowes, Hilliard, OH	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
Unpainted Concrete	ASTM C90 cinder block	Wellnitz, Columbus, OH	1.9 cm x 7.6 cm x 0.2 cm	Autoclave

5.4 Inoculation of Coupons

Test and positive control coupons were placed on a flat surface within a Class II biological safety cabinet (BSC) and inoculated with approximately 1×10^8 CFU of viable *B.g.* spores per coupon. A 100 microliter (µL) aliquot of a stock suspension of approximately 1×10^9 CFU/mL was dispensed using a micropipette applied as 10 µL droplets across the coupon surface (Figure 5-2). This approach provided a more uniform distribution of spores across the coupon surface than

would be obtained through a single drop of the suspension. Although application of the inoculum onto each material was uniform, the behavior of the inoculum droplets was not. Droplets beaded on the surface of the ceramic tile (nonporous material) while they soaked into the unpainted

concrete materials after producing a liquid bead for a short period. The difference in the behavior of the inoculum droplets on each material could lead to a variance in microorganism distribution across coupons; however, this effect was not studied in this evaluation. After inoculation, the coupons were left undisturbed overnight to dry under ambient conditions, approximately 22 °C and 40% RH.

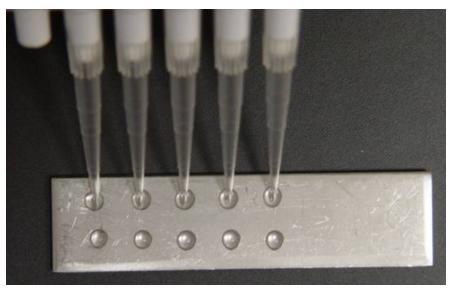


Figure 5-2 Liquid inoculation of coupon using a micropipette.

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

- Three test coupons (inoculated with *B. atrophaeus* spores and sprayed with sporicidal liquid)
- Three positive controls (inoculated with *B. atrophaeus* spores and sprayed with water)
- One laboratory blank (not inoculated and not sprayed with sporicidal liquid)
- One procedural blank (not inoculated and sprayed with sporicidal liquid).

On the day following inoculation, coupons intended for decontamination (including blanks) were transferred to the ambient breeze tunnel (ABT) test facility, placed in one of two designated positions, and exposed to the pAB using the AOF apparatus and application conditions specified in Section 2.5. Details of coupon handling can be found in Section 5.6.

5.5 Spraying Equipment

Figure 5-3 is a photo of the modified AOF 2-36 orchard sprayer used for both the field scale demonstration and efficacy testing. Although a larger D-40R model was selected in the market survey portion of the study, due to weight and size limitations for the field scale demonstration cart, a few modifications were necessary along with selecting the smaller sized D2-36 model. Modifications included removal of the pneumatic wheels (unit to be mounted on rail car), exchange of diesel engine for V10 aluminum block gasoline engine, smaller 250-gallon stainless

steel tank, and an added 3.7 m downward facing spray bar. The orchard sprayer fluidics were controlled by an AgOtter controller (AgOtter, Tempe, AZ) which uses global positioning systems and axle mounted monitors to determine speed and location of the equipment. This device continuously adjusts a series of valves to ensure the target surfaces are getting an equal amount of liquid deposition regardless of ground speed. The 2-stage 200 pounds per square inch (psi) centrifugal



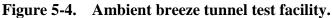
Figure 5-3. Customized AOF Model 2-36

myers pump supplied the water or pAB to 85 Teejet ceramic conejet (TeeJet Technologies, Glendale Heights, IL Model# TXR800013) nozzles including 12 that were downward facing.

5.6 Ambient Breeze Tunnel and Procedures

Decontamination testing was conducted inside Battelle's ABT test facility located in West Jefferson, Ohio, which has height and width dimensions of similar scale to many subway tunnels (20 feet W x 20 feet H x 135 feet L). Figure 5-4 shows the exterior of the ABT test facility. The ABT has an upstream and downstream blower exhaust system that was operated during each test to achieve an approximately 1.5 mph cross wind. This not only replicated wind that would be generated by traversing through the subway at 1.2 mph, it also minimized the amount of wraparound from the pAB to the engine and operator area of the test equipment. Once testing had concluded each day, all exhaust blowers were turned off to minimize any enhanced drying effect from the excess air movement.





Due to the large size and weight of the AOF sprayer, it was determined that controlling movement of the test coupons would be more efficient than controlling the AOF sprayer. A custom cart was designed and fabricated that would allow test coupons to be held at 5 different orientations to the spray plume (Figure 5-5). These locations would be representative of the tracks on the floor and all four sides of a pillar commonly found in a terminal platform location. At each location, wetness was measured in terms of total percent coverage using a HOBO[®] S-

LWA-M003 leaf wetness sensor connected to a HOBO H21-002 micro station data logger (Onset, Bourne, MA), which recorded wetness measurements every ten seconds for the duration of each spray application. Two test positions were selected that exhibited the lowest percent wetness from an average of three tests. These positions included one vertical right side of column position (location 1), and one horizontal floor (location 2).



Figure 5-5. AOF sprayer and test cart configuration.

Testing targeted 10 °C to replicate conditions commonly found in underground subway tunnels and platforms. Due to the scale of testing (field scale), temperature was not controllable and therefore tests were planned for days that met temperature requirements, which resulted in higher

than normal variability. Temperature and RH in the ABT were measured every minute during experimental exposure using an HOBO MX temp/RH data logger.

Each test consisted of coupon inoculation the afternoon before testing was to occur. The following day, those test coupons were transferred to the ABT testing facility and the control coupons and blanks were loaded into test positions and held in place by metal clips. Once operational test temperatures were observed the sprayer was operated with water and the test cart was moved through the spray plume at the desired speed using a 1 horse power variable speed DC motor (Dayton Manufacturing Co., Niles, IL) over a 15.2 m aluminum track. Control coupons and laboratory blanks were then collected and placed into clean 50 mL conical tubes and kept in the same orientation (vertical/horizontal) as they were sprayed. The caps of the conical tubes were left open during the exposure to maintain evaporative effects. Each coupon platform was then washed with bleach followed by ethanol wipe to minimize any carry over of viable control organism to test coupons. The test coupons and procedural blanks were then placed onto the test cart and the pAB was prepared within the AOF equipment. Aliquots were removed to ensure proper concentration and pH of the prepared pAB. The pH was measured using a handheld Thermo Seven-Go pH meter (Thermo Scientific, Waltham, MA). pAB ppm was measured an iodometric determination of chlorine dioxide and chlorite using a HACH[®] test kit (HACH, Loveland, CO). The test cart was then traversed through the spray plume and coupons collected in an identical manner as the controls. The test and control coupons were held in the ABT for the defined contact time per test.

5.7 Coupon Extraction and Biological Agent Quantification

Spore extraction was achieved by placing test, positive control, and blank coupons in 50 mL polypropylene conical tubes containing 10 mL of sterile phosphate buffered saline with 0.1% TritonTM x-100 [Sigma-Aldrich, St. Louis, MO (PBST)]. The vials were capped, placed on their side and agitated on an orbital shaker for 15 minutes (min) at approximately 200 revolutions per minute (rpm) at room temperature.

The amount of residual viable spores was determined using a dilution plating approach. Following extraction, the extract was removed, and a series of tenfold dilutions was prepared in sterile filtered water. An aliquot (0.1 mL) of either the undiluted extract and/or each serial dilution was plated onto tryptic soy agar in triplicate and incubated for 18 to 24 hours at 37 ± 2 °C. Colonies were counted manually and CFU/mL was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Dilution data representing the greatest number of individually definable colonies were expressed as arithmetic mean \pm standard deviation (SD) of the numbers of CFU observed. Laboratory blanks controlled for sterility and procedural blanks controlled for viable spores that could have been inadvertently introduced to test coupons. The target acceptance criterion for extracts of laboratory or procedural blanks was zero CFU.

After each decontamination test, the test cart was thoroughly cleaned (using separate steps involving bleach, ethanol, then drying).

5.8 Decontamination Efficacy

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

Mean % Recovery = [Mean
$$CFU_{pc}/CFU_{spike}$$
] × 100 (1)

where Mean CFU_{pc} is the mean number of CFU recovered from three replicate positive control coupons of a single material, and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} was known from enumeration of the stock spore suspension. One aliquot of the stock suspension was plated and enumerated on each day of testing to confirm CFU_{spike} concentration. Spore recovery was calculated for *B.g.* on each coupon, and the results are included in Section 7 and Appendix A.

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

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

$$Efficacy (LR) = (\log_{10} CFUc_{ii}) - (\log_{10} CFUt_{ii})$$
(2)

where $\log_{10} CFUc_{ij}$ refers to the *j* individual logarithm values obtained from the positive control coupons and $\log_{10} CFUt_{ij}$ refers to the *j* individual logarithm values obtained from the individual corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were three positive controls and three corresponding test coupons (i.e., *j* = 3) for each coupon. A decontaminant or fumigant technology is considered to be effective via AOAC test method 966.04 if a 6 LR or greater is achieved (1).

In the case where no viable spores were found in any of the three test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a \log_{10} CFU of 0 for that material. This situation occurred when the decontaminant was highly effective, and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to (\geq) the value calculated by Equation 2.

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

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

where the number 3 again represents the number j of coupons in both the control and test data sets. Each efficacy result is reported as an LR value with an associated 95% confidence interval (CI), calculated as follows:

95 % CI = Efficacy (LR)
$$\pm$$
 (1.96 \times SE) (4)

The significance of differences in efficacy across different test conditions and spore types was assessed based on the 95% CI of each efficacy result. Differences in efficacy were judged to be

significant if the 95% CIs of the two efficacy results did not overlap. Any results based on this formula are hereafter noted as significantly different. Note this comparison is not applicable when the two efficacy results being compared are both reported with LRs as \geq some value.

5.9 Surface Damage

The physical effect of the sporicidal liquids as delivered by the AOF equipment on the materials was qualitatively monitored during the evaluation. This approach provided a gross visual assessment of whether the environmental state changed the appearance of the test materials. The procedural blank was visually compared to a laboratory blank coupon.

6.0 Quality Assurance/Quality Control

Quality assurance (QA) and quality control (QC) procedures were performed in accordance with with EPA ORD's QA program and Battelle's QA program. The QA/QC procedures and results are summarized below.

6.1 Equipment Calibration

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

6.2 QC Results

QC efforts conducted during decontaminant testing included positive control samples procedural blanks, laboratory blanks, and inoculation control samples.

Positive control results were in many cases lower than the target recovery range of 5 to 120% of the inoculated spores. Recoveries ranged from 3.55% to 15.3%, and 0.13% to 3.24% from ceramic tile and unpainted concrete, respectively. Low recoveries from unpainted concrete are not uncommon due to the porosity of the materials; however, the low recoveries from the ceramic tile were most likely due to mechanical removal of spores from the coupons as observed by re-deposition and enumeration of target bacterial colonies from blank materials of both unpainted concrete and ceramic tile. LRs of >6 were achievable in most instances, even with the low recoveries.

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

6.3 **Operational Parameters**

The temperature, RH, and wetness for each test was monitored as described in Section 5.0. For all tests, the temperature and relative humidity was uncontrolled but monitored as described in Section 5.6. Testing was scheduled based on weather forecasting and not initiated until minimum of 7.2 °C was achieved. Readings were taken once every minute for the duration of the spray and contact time. The percent wetness was measured for all 5 locations (only target locations reported) every 10 seconds for the duration of the spray and up to 15 min post application. The actual operational parameters for each test are shown in Table 6-1 and reported as the average value \pm SD.

Test Number -	Avg. Control Wetness		Avg. pAB Wetness		Temperature (°C)		RH (%)		Contact Time
	Location #1	Location #2	Location #1	Location #2	Target	Actual*	Target	Actual*	(hours)
1	30.2 ± 3.81	98.0 ± 10.9	43.4 ± 0.83	98.3 ± 3.33	10	15.5 ± 1.17	None	59.1 ± 3.93	0.5
2	27.1 ± 5.23	72.5 ± 10.2	45.4 ± 4.58	84.8 ± 7.87	10	12.7 ± 1.41	None	79.7 ± 7.74	12^{\dagger}
3	37.1 ± 2.40	54.1 ± 4.12	43.3 ± 11.0	57.1 ± 20.5					
4a	27.5 ± 2.85	62.3 ± 14.5	30.0 ± 1.38	48.5 ± 1.80					
4b	26.9 ± 5.66	47.5 ± 4.39	55.2 ± 3.82	72.7 ± 3.37	10	11.2 ± 1.26	None	98.0 ± 4.94	10^{\dagger}
4c	36.7 ± 4.36	60.1 ± 5.92	63.9 ± 4.71	66.5 ± 5.70					

Table 6-1. Actual Operational Conditions for Tests

* Data reported as average \pm SD.

[†] Overnight contact time, samples were not staggered and thus value is approximate.

6.4 Audits

6.3.1 Performance Evaluation Audit

Performance evaluation (PE) audits were conducted to assess the quality of the results obtained during these experiments. Table 6-2 summarizes the PE audits that were performed.

No PE audits were performed for confirmation of the concentration and purity of *B.g.* spores because quantitative standards do not exist for this organism. The titer enumerations and the control and blank test coupons support the spore measurements.

 Table 6-2.
 Performance Evaluation Audits

Measurement	Audit	Allowable	Actual	
wieasui ement	Procedure	Tolerance	Tolerance	
Volume of liquid from	Gravimetric evaluation	+ 10 %	± 0.8 % to 3.0 %	
micropipettes	Gravimente evaluation	± 10 70	± 0.8 % to 3.0 %	
Time	Compared to independent clock	± 2 seconds/hour	0 seconds/hour	
Tomporatura	Compared to independent calibrated	± 2 °C	± 0.58 to 1.73 °C	
Temperature	thermometer	±2 C		
Relative Humidity	Compare to independent calibrated	+ 10 %	± 0.04 to 0.67 %	
	hygrometer	± 10 70		

6.3.2 Technical Systems Audit

Observations and findings from the technical system audit were documented and submitted to the laboratory technical lead for response. The audit was conducted on November 29, 2016 to ensure that tests were conducted in accordance with the QAPP. As part of the audit, test procedures were compared to those specified in the QAPP and data acquisition and handling procedures were reviewed. None of the findings of the audit required corrective action.

6.3.3 Data Quality Audit

At least 10 % of the data acquired during the evaluation were audited. Data was reviewed in one batch February 2017. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All

calculations performed on the data undergoing the audit were verified. Only minor issues were noted with the data, mostly data transcription errors that were corrected.

6.5 QA/QC Reporting

Each assessment and audit was documented in accordance with EPA ORD's QA program and Battelle's QA program. For these tests, findings were noted (none significant) in the data quality audit, and no follow-up corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. QA/QC procedures were performed in accordance with EPA ORD's QA program and Battelle's QA program.

6.6 Data Review

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

7.0 Results and Discussion

A survey of commercially available or fielded equipment was conducted in order to inform remediation efforts following a large-scale contamination incident involving an underground transportation system. A working group, comprised of EPA, EPA's technical support contractor for this effort (Battelle) and stakeholders representing Transit Authority's then ranked the identified equipment based upon criteria pertinent to the equipment's use during a biological remediation within a subway system. The three highest pieces of identified pieces of equipment (MM Sprayers, AOF, and Dust Boss sprayers) were subjected to durability tests, to evaluate their compatibility with pAB. These tests included 100 hours of operation with pAB using smaller proxy equipment designed in consultation with the vendors of the equipment. Based on durability assessment two pieces of equipment (AOF and Dust Boss sprayers) were further down-selected to participate in a field scale demonstration at a subway platform/tunnel at Fort A.P. Hill. The equipment was placed atop a flatbed railcar and water was sprayed through the subway platform/tunnel at a speed of 1.2 mph while video and leaf wetness data (5 locations) were collected. Based on the leaf wetness data, review of video, and observer input (Appendix D), a single piece of equipment was selected (AOF) to perform field-scale efficacy tests using B.g. spores as a surrogate for B. anthracis.

Efficacy testing was conducted within Battelle's ABT testing facility that permitted full-scale implementation as the internal dimensions of this facility were representative of many existing subway tunnels. Decontamination efficacy of operationally sprayed pAB against *B.g.* was evaluated at target delivery speeds of 1.2 and 2.4 mph, target temperature of 10 °C, uncontrolled RH ranging from 59 to 98%, coupon orientations simulating floor and column (right side) and contact times ranging from 30 min to 12 hours (overnight) for a total of 5 tests. Ceramic tile resulted in ≥ 6 LR at each condition tested (tests 1-3). Unpainted concrete resulted in LRs ranging from 1.62 to 2.34 and 1.32 to 3.02 at locations 1 and 2, respectively. Since concrete was

more challenging, Test 4 utilized concrete only with repeat applications (2 and 3) with 30 min contact times between applications. This resulted in increased LR ranging from 2.95 to 4.70. Actual operational parameters as measured were within acceptable ranges and are detailed in Section 5. The detailed decontamination efficacy results are found in Appendix A.

7.1 Durability Testing

Results comparing performance of the three downselected equipment from the market survey (MM Sprayer, AOF, and Dust Boss sprayers) operated with pAB are shown in Figures 7-3 and 7-4. After four hours of operation both the MM Sprayer and Dust Boss pAB test equipment were found to have



Figure 7-1. MM sprayer failure.

failed while the control equipment performed as anticipated. Further investigation found that the MM sprayer diaphragm pump had failed as noted by lack of operational pressure when found due to a ruptured seal as well as the evacuation of pump oil from the reservoir as shown in Figure 7-1. The vendor was consulted and provided replacement seal part numbers. The Dust

Boss sprayer had also failed as noted by a sharp increase in liquid flow rate as shown in Figure 7-3. Upon investigation, it was found that the pAB had a corrosive effect on the brass nozzle and had increased the orifice size as shown in Figure 7-2. The vendor was consulted and determined that a stainless-steel version (part # 64-000131A) of the provided nozzle would likely result in increased durability, though this nozzle was not used for this testing.



Figure 7-2. Dust Boss nozzle failure. From left: control, test 1 test 2 and test 3 nozzles.

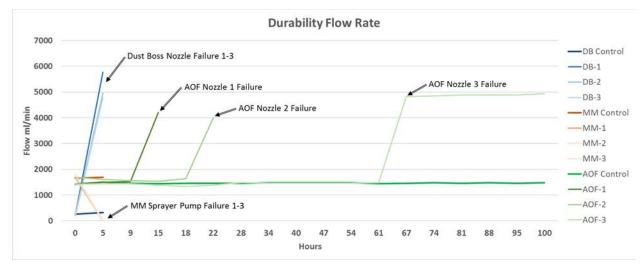


Figure 7-3. Durability test flow rate.

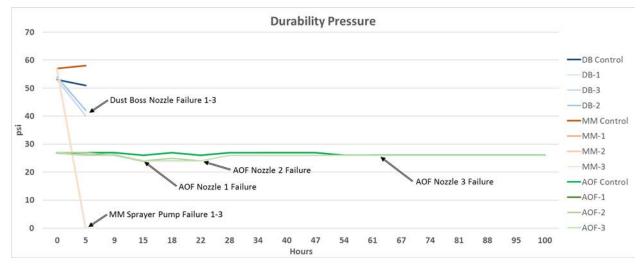


Figure 7-4. Durability test pressure.

The AOF system performed the longest with the first nozzle failure occurring after 15 hours of operation followed by nozzle 2 failure at 22 hours and nozzle 3 failure at 67 hours as noted by decreased pressure and increased flow rate. Upon investigation of each nozzle failure it was found that the plastic nozzle orifice housing had become brittle and broken off as seen if Figure 7-5. Testing was continued out to 100 hours with the AOF pump only which resulted in no observable deterioration of flow rate or pressure. The vendor was consulted and determined that using a standard ceramic disc and



Figure 7-5. AOF nozzle failure. From left: control, test 1, test 2, and test 3 nozzles.

core nozzle would eliminate the observed failure, though this nozzle was not used for this testing. Further details on the durability test parameter results are found in Appendix B.

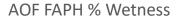
7.2 Field Scale Demonstration

Results comparing water deposition rate performance of the AOF and Dust Boss equipment operated with water at a speed of 1.2 mph are shown in Figures 7-6 and 7-7. The AOF sprayer delivered approximately 908 L of water over the entire distance of the subway system (~197 m) in approximately 6 min at a flow rate of 151 lpm. The radial placement of the nozzles in addition to the added down-facing spray bar resulted in 100% max coverage of all wetness sensors as noted in Table 7-1. Video collected during testing showed the wicking effect of the applied liquid into the concrete walls. Over the course of approximately 12 min large portions of the walls changed from visually dark color (saturated) to a much lighter color indicating a drying effect.

The Dust Boss sprayer delivered approximately 189 L of water over the entire distance of the subway system in approximately 6 min at a flow rate of 32 lpm. The design of the Dust Boss sprayer resulted in a more focused application of liquid and was only able to achieve 100% coverage at one location. While an individual DB30 system may not be adequate for full coverage of a subway tunnel and platform, adding multiple DB30 system may result in the desired coverage. The temperature and relative humidity during testing ranged from 16.7 to 18.2 °C and 75 to 93% respectively.

Equip	oment					
		Railing	Tunnel Wall	Column (Back)	Ceiling	Tracks
Air-O-Fan	Average	56.7	34.3	93.4	95.6	90.1
All-O-Fall	Max	100.0	100.0	100.0	100.0	100.0
Dust Poss	Average	83.2	20.9	42.1	27.3	25.4
Dust Boss	Max	100.0	37.1	68.8	47.7	48.8

Table 7-1. Fort A.P. Hill Subway Percent Wetness



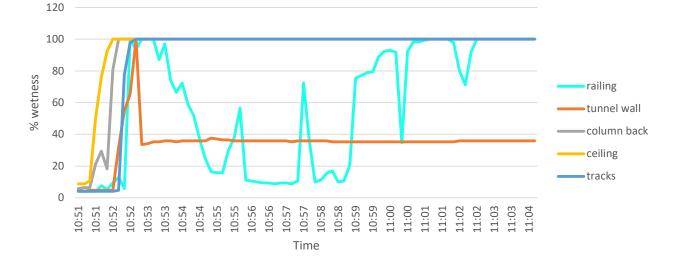


Figure 7-6. AOF sprayer Fort A.P. Hill (FAPH) demonstration % wetness.

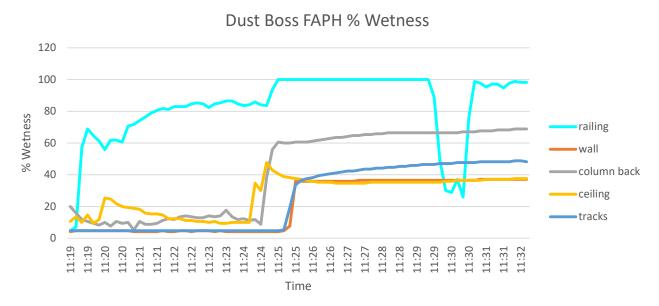
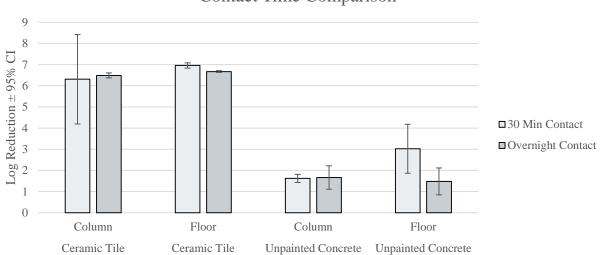


Figure 7-7. Dust Boss sprayer Fort A.P. Hill (FAPH) demonstration % wetness.

7.3 Efficacy Testing

The decontamination efficacy of pAB delivered via the AOF orchard sprayer against *B.g.* was evaluated on ceramic tile and unpainted concrete at two locations, contact times of 30 min or overnight, delivery speeds of 1.2 and 2.4 mph, and repeat applications of 1, 2 and 3 with a 30 min contact time between applications. A target temperature of 10 °C was used to represent the ambient environmental conditions that would be expected in underground subway platforms and tunnels.

Results are organized by test condition in Figures 7-8 through 7-11 to visualize the effect of contact time, operational speed, and repeat application of pAB and water, respectively. Figure 7-8 indicates little to no difference exists between contact times of 30 min and 12 hours (overnight). Similarly, as shown in Figure 7-9, little difference is seen between spray delivery speeds of 1.2 and 2.4 mph. Both tests show significant differences between Ceramic Tile and Unpainted Concrete with average LR of 6.8 and 1.9, respectively.



Contact Time Comparison

Figure 7-8. 30 min (Test 1) vs overnight contact time (Test 2).

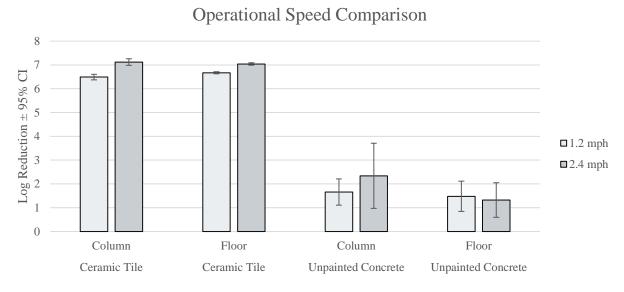


Figure 7-9. 1.2 mph (Test 2) vs 2.4 mph application rate (Test 3).

Since the first three tests all resulted in greater than 6 LR on ceramic tile, Test 4 was conducted with unpainted concrete only. Three repeat applications of pAB each with a 30 min contact in between resulted in an upward trend of LR with both 2 and 3 applications being significantly higher than 1 at location 1. In addition, the physical removal of spores by multiple water applications (Controls) from concrete can be observed in Figure 7-11 and is supported by the redeposition of *B.g.* colonies onto the procedural blanks. Little difference can be observed when comparing 1 through 4 applications of water at location 2, however an increased recovery is noted at location 1 for 2, 3 and 4 applications possibly due to rehydration of the spore and subsequent loosening from the substrate. LR data for each specific material, are included in Appendix A.

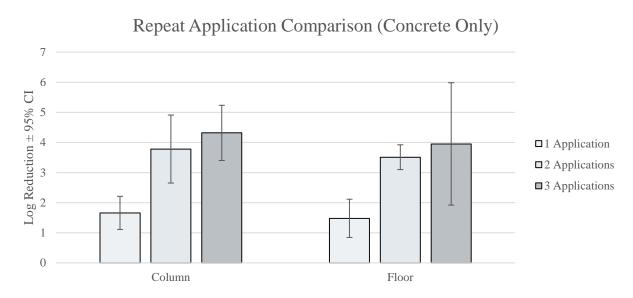
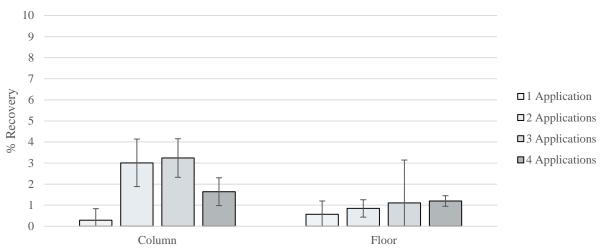


Figure 7-10. Test 2, 4a, and 4b comparison.



1.2 mph Mechanical Removal on Concrete

Figure 7-11. Test 2, 4a, 4b, and 4c comparison.

7.4 Surface Damage to Materials

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

7.5 Summary

This evaluation focused on the identification and evaluation of commercially-available equipment for the spraying of sporicidal liquids in a subway environment. Three equipment types were down-selected from a market survey producing 22 total candidate technologies. Durability testing was conducted on the top three to determine material compatibility of each equipment's wetted components. Although none of the three reached 100 hours of testing without some level of failure, two technologies (AOF and Dust Boss) determined that parts exist that could extend use in future testing or actual implementation. These two technologies were also used for a field-scale demonstration at FAPH in which water was sprayed through the subway tunnel/platform. Wetness data showed that the current configuration of the AOF equipment was suitable for equal distribution of liquid through both the tunnel and platform sections as evidenced by 100% coverage wetness data at each of 5 test locations. It was noted that the Dust Boss sprayer performed well, but multiple units would need to be configured to achieve more complete coverage. It is estimated that a minimum of four DB30 units would be required to attain complete coverage of subway tunnels and platform sections. Additional decontamination and spray distribution testing is needed to confirm this estimate.

Based on performance in durability and field-scale demonstration testing the AOF sprayer was selected for field-scale efficacy testing. Decontamination efficacy of operationally-sprayed pAB

against *B.g.* was evaluated at target delivery speeds of 1.2 and 2.4 mph, target temperature of 10 °C, uncontrolled RH ranging from 59 to 98%, coupon orientations simulating floor and column (right side), and contact times ranging from 30 min to 12 hours (overnight) for a total of 4 tests. Since efficacy was not affected by speeds up to 2.4 mph (25 min/mile) nor contact times longer than 30 minutes, it can be estimated that one AOF unit could deliver three applications of pAB (151 lpm) per mile of subway tunnel/platform in 2 hours and 45 minutes (25 min application time, 30 min contact time, per application). These conditions achieved complete inactivation on ceramic tile and ~4 LR on unpainted concrete during testing. This application regimen would use 11,325 L of decontaminant per mile of track.

Over the course of the study all testing conducted with ceramic tile resulted in > 6 LR of *B.g.*, while no conditions were found that resulted in > 6 LR of *B.g* on unpainted concrete. It was found that neither decontamination delivery speeds of 1.2 or 2.4 mph, nor increased contact times greater than 30 min resulted in a significant effect on LR. Repeat applications of pAB up to three each resulted in increased efficacy.

This work provides several candidate technologies that could be useful during remediation following a wide area release of *B. anthracis*, specifically in a subway environment. This study also provides information on the efficacy of pAB as delivered by the top selected equipment (AOF) against surrogate B.g. spores for decontamination of common subway materials that could become contaminated with *B.a.* spores. Such results may be useful in the development of guidance to aid in deployment of sporicidal liquid after a wide-area release of *B.a.* spores in a subway environment.

Note: See Appendix E for additional results from add-on testing.

8.0 References

- 1. U.S. Environmental Protection Agency. Determining the Efficacy of Liquids and Fumigants in Systematic Decontamination Studies for *Bacillus anthracis* Using Multiple Test Methods. Research Triangle Park, NC: U.S. Environmental Protection Agency. US EPA Report 600/R-10/088, December 2010.
- 2. Calfee MW, Ryan SP, Wood JP, Mickelsen L, Kempter C, Miller L, Colby M, Touati A, Clayton M, Griffin-Gatchalian N, McDonald S, Delafield R, *Laboratory evaluation of large-scale decontamination approaches*. J. Applied Microbiology, May 2012; 112(5): 874-82

Appendix A Detailed Test Results

Efficacy Results

The detailed decontamination efficacy results for pH amended bleach (pAB) sprayed with Air-O-Fan (AOF) against *Bacillus atrophaeus* on Two material types are shown in Table A-1. Colony-forming units (CFU) were observed on all procedural blanks indicating mechanical removal and redeposition of spores.

Test							Mean Recovere	ed B. atrophaeus	
Number							Positive Control ^b	Test Coupon ^c	
					Ceramic Tile L1		$2.49 \pm 0.40 \; x \; 10^7$	$5.67 \pm 9.81 \ x \ 10^2$	6.31 ± 2.11
		105	0.5	10	Ceramic Tile L2	1.005.00	$9.42 \pm 2.51 \; x \; 10^6$	0.00 ± 0.00	${\geq}6.96\pm0.12$
1	pAB	AOF	0.5	10	Unpainted Concrete L1	1.99E+08	$7.93 \pm 1.03 \; x \; 10^5$	$1.99 \pm 0.78 \; x \; 10^4$	1.62 ± 0.20
					Unpainted Concrete L2		$5.69 \pm 7.55 \; x \; 10^6$	$5.90 \pm 6.09 \ x \ 10^3$	3.02 ± 1.16
					Ceramic Tile L1		$3.13 \pm 0.71 \; x \; 10^6$	0.00 ± 0.00	${\geq}6.49\pm0.12$
		105	10	10	Ceramic Tile L2	0.007.07	$4.65\pm 0.40\;x\;10^{6}$	0.00 ± 0.00	$\geq\!\!6.67\pm0.04$
2	pAB	AOF	12	10	Unpainted Concrete L1	8.80E+07	$2.53 \pm 1.31 \; x \; 10^5$	$6.12 \pm 4.00 \ x \ 10^3$	1.66 ± 0.55
					Unpainted Concrete L2		$5.06 \pm 0.45 \; x \; 10^5$	$2.76 \pm 3.03 \ x \ 10^4$	1.48 ± 0.63
					Ceramic Tile L1		$1.35\pm 0.38\ x\ 10^{7}$	0.00 ± 0.00	${\geq}7.12\pm0.14$
2		105	10	10	Ceramic Tile L2	0.007.07	$1.09\pm 0.11\ x\ 10^{7}$	0.00 ± 0.00	${\geq}7.04\pm0.05$
3	pAB	AOF	12	10	Unpainted Concrete L1	8.80E+07	$1.64 \pm 0.36 \; x \; 10^5$	$2.92 \pm 3.68 \; x \; 10^3$	2.34 ± 1.37
					Unpainted Concrete L2		$1.17 \pm 0.53 \; x \; 10^5$	$8.34 \pm 6.45 \ x \ 10^3$	1.32 ± 0.73
	1.5	105	0.5	10	Unpainted Concrete L1	0.007.07	$2.98 \pm 1.86 \ x \ 10^6$	$1.46 \pm 2.26 \; x \; 10^3$	3.78 ± 1.13
4a	pAB	AOF	0.5	10	Unpainted Concrete L2	9.90E+07	$8.43 \pm 2.06 \; x \; 10^5$	$3.22 \pm 2.71 \ x \ 10^2$	3.51 ± 0.41
				10	Unpainted Concrete L1	0.00 P 0 ⁻	$3.21 \pm 2.80 \ x \ 10^6$	$3.00 \pm 4.33 10^2$	4.32 ± 0.92
4b	pAB	AOF	0.5	10	Unpainted Concrete L2	9.90E+07	$1.10 \pm 0.50 \; x \; 10^6$	$8.34 \pm 7.54 \; x \; 10^2$	3.95 ± 2.03
	1.5		10	Unpainted Concrete L1	0.007.07	$1.62\pm 0.66\;x\;10^{6}$	$1.67 \pm 0.85 \ x \ 10^3$	2.95 ± 0.36	
4b	4b pAB	AOF	0.5	10	Unpainted Concrete L2	9.90E+07	$1.19 \pm 0.25 \; x \; 10^6$	$4.30 \pm 7.45 \ x \ 10^3$	$4.70\pm~2.69$

^a Data are expressed as the mean (\pm SD) of the logs of the number of spores (CFU) observed on three individual samples and decontamination efficacy (log reduction).

^bPositive Controls = samples inoculated, not decontaminated.

^c Test Coupons = samples inoculated, decontaminated.

 d CI = confidence interval (± 1.96 × SE).

Appendix B Detailed Durability Results

Durability Results

The detailed durability results for pH-amended bleach (pAB) sprayed with MM Sprayers, Dust Boss sprayer, and AOF proxy systems are shown in Tables B-1 and B-2.

Table B-1. Durability Testing Pressure

														Pressu	ure Me	easure	ment	s (psi)																
Equipment	5	hr	9	hr	1	ōhr	18	3hr	22	2hr	28	hr	34	4hr	4(Dhr	47	7hr	54	1hr	61	lhr	67	7hr	74	4hr	8	1hr	88	3hr	9	5hr	10	00hr
DB Control	53	51																																
DB-1	54	42*																																
DB-2	54	42*																																
DB-3	53	40 *																																
AOF Control	27	27	27	27	27	26	27	27	27	26	27	27	27	27	27	27	27	27	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
AOF -1	27	26	26	26	26	24*																												
AOF -2	27	27	26	26	26	24	24	25	24	24*																								
AOF -3	27	26	26	26	26	24	24	24	24	24	26 ⁺	26	27	26	26	26	26	26	26	26	26	26	26	26*	26	26	26	26	26	26	26	26	26	26
MM Control	57	58																																
MM-1	57	0*																																
MM-2	57	0*																																
MM-3	57	0*																																

*indicates equipment found failed.

†pressure adjusted to maintain operational conditions with reduced number of functional nozzles.

Table B-2. Durability Testing Flow

						FI		Measureme)								
Equipment	5	ihr	9	hr	1	15hr		8hr	22	hr	28	hr	34	hr	40)hr	47	7hr
DB Control	260	320																
DB-1	240	5770 [*]																
DB-2	240	4950 [*]																
DB-3	190	4770 [*]																
AOF Control	1420	1500	1480	1450	1460	1440	1480	1460	1480	1460	1460	1460	1460	1480	1480	1480	1500	1480
AOF -1	1440	1500	1500	1520	1470	4200 [*]	4260	4650	4680									
AOF -2	1690	1610	1540	1560	1540	1540	1620	1630	1600	4000*								
AOF -3	1420	1440	1430	1430	1400	1380	1380	1340	1320	1380	1450	1480	1440	1460	1500	1460	1440	1460
MM Control	1660	1690																
MM-1	1690	0*																
MM-2	1780	0*																
MM-3	1780	0*																

*indicates equipment found failed.

Table B-2. Durability Testing Flow (Continued)

Flow Rate Measurements (mL/min)																
Equipment	54	1hr	6	1hr	67hr		74hr		81	hr	88	hr	95	ihr	10	0hr
DB Control																
DB-1																
DB-2																
DB-3																
AOF Control	1500	1480	1420	1440	1460	1460	1460	1480	1450	1460	1480	1480	1480	1460	1460	1480
AOF -1																
AOF -2																
AOF -3	1460	1460	1440	1480	1480	4820 [*]	4820	4850	4900	4880	4850	4880	4920	4890	4900	4940
MM Control																
MM-1																
MM-2																
MM-3																

*indicates equipment found failed.

Appendix C Detailed Market Survey Results

Table C-1. Commercial Equipment for Subway Decontamination

	Company	Model #	Flow Rate	Engine/ PTO	Diameter of fan		Spray Distance	Weight	Width X Length	Height	Horse Power/Fuel Tank Size	Tank Material	Nozzle Info/Droplet size (micron)	Price	Unique Features	Country of Origin
	Nelson Hardie Mfg, Co., Inc.	6800P	10-25 GPM	engine and PTO available	Dual 34" fan PTO drive	2	40'	500 Gal. 3,500lbs, 1000Gal. 4,100lbs.	102 x 241"	75-85"	70-100PTP HP/540 RPM max	12 ga., Type 304 stainless steel	t-jet ceramic disk nozzle/ 50- 200	- 33-37k	radial spray pattern, myers pump,	Yuba City, CA, USA
	Nelson Hardie Mfg, Co., Inc.	Super 92	10-25 GPM	engine and PTO available	Dual 46" fan, 325 HP engine drive	2	80'	9,080 Lbs. (Empty)	102 x 322"	75-85"	150 Gallon tank 325Hp	12 ga., Type 304 stainless steel	50-200	100-120K	radial spray pattern	Yuba City, CA, USA
Radial Fan	M.K. Rittenhouse & Sons Ltd.	PRM1500- ATPE	37 GPM (140.1 LPM), 725 PSI (50 bar)	РТО	35.5" (90 cm) Fan	1	30-40'	1200lbs.	54 x 152"	82"	395 gal	polyethylene	unknown t- jet ceramic disk nozzle	\$10,250.00	14 double-sided flipover nozzles	Buffalo, NY, USA
Sprayers	Air-O-fan Products Corp.	D-40R 1,000 Gallon	1-100 GPM at 200 PSI, Yes	engine and PTO available	40" steel axial flow "reverse" fan	1	180-200'	8,460 pounds	105 x 246"	62-72"	156 HP	stainless steel	60-100	60-100K	radial spray pattern	Reedley, CA, USA
	MM Sprayers USA	MM LG 400	yes	engine and PTO available	25"	1	20'	583 lbs Dry	45 x 100"	54"	13 HP	106 gal poly	t-jet	9,437.00	radial spray pattern	Lynden, WA, USA
	Nobili	Euro-T	yes	РТО	single fan with directed hoses	1	unknown	281-570lbs	69x192"	69"	300-4000 L tank	fiberglass	varies	\$428-\$36082	resists chemicals used for pesticide treatments	Molinella, Italy
	Nobili	Oktopus P/T, various	yes 81 to 110 L/1'	РТО	single fan with directed hoses	1	unkown	281-570lbs	63x142"	79-142"	1500 L	polyethylene tank	varies	\$8539-\$14834	Self cleaning filters	Molinella, Italy
	Vinetech equipment	Quantum Mist Citrus Sprayer	1.2 to 20 GPM per fan	РТО	15 or 20"	scaleable	10'	varies	15 and 20" fans available	NA - fans configured on cusstom frame	hydraulic drive	NA	50-80 microns	\$1480-\$1680 per fan	scalable	Prosser, WA, USA
Air Directed Sprayers	Nixalite	Nightstar 1901 ULV/LVM	0.5 - 4.1 GPH	electric 115V 19 AMP	22" DIAMETE R FAN	1	treats up to 60K ft ²	220 LBS EMPTY / 252 LBS FULL	38 X 32"	81"	ELECTRIC MOTOR, 1 HP, 3 gal	polyethylene, FLUSH TANK IS HDPE		\$7,300.00 PLUS SHIPPING & HANDLING	Wetted Parts material HDPE, BRASS AND VITON SEALS	East Moline, IL, USA
	Bigassfans	AirGO	Air velocities range from 528 fpm to 113 (to 140') fpm	electric 120V 5A	8'	NA	NA	105 lb (47.6 kg)	96" round	96" when vertical	No tank	Blades: High performance polyamide nylon	~10 microns	9,000.00	NA	Lexington, KY, USA
	Leader	Easy 4000	260 LPM	engine	~40	1	200'	372 kg	43 x 58"	63"	42L	NA	NA	40-100K	sprays foams	Mooresville, NC, USA

	Company	Model #	Flow Rate	Engine/ PTO	Diameter of fan		Spray Distance	Weight	Width X Length	Height	Horse Power/Fuel Tank Size	Tank Material	Nozzle Info/Droplet size (micron)	Price	Unique Features	Country of Origin
Electrostatic Sprayers	Electrostatic Spraying Systems	100SR	yes, 2.88-5.03 LPM	РТО	sprayers use blowers	1	unknown	600 lbs dry	custom fabricated based on specific needs.	custom fabricated based on specific needs.	Requires 65 HP tractor minimum	Stainless Steel	40 micron droplet size	\$29,000 to \$31,000	electrostatic nozzles	St Watkinsville, GA, USA
	Dust Boss	DB-30	1.4 - 2.8 GPM	Electric	9200 CFM	1	100'	800 pounds	63 x 102"	64"	Does not have a tank	Does not have a tank	Anywhere from 50 to 200 microns	Rental of a DB-30 would be 875 per week	0-70° or 0 to 359° oscillation	Peoria, IL, USA
	Dust Boss	DB-45	Average of around 12 GPM	Electric	1800 CFM	1	150'	1800 pounds	72 x 76"	82"	Does not have a tank	Does not have a tank	Anywhere from 50 to 200 microns	Rental of a DB-45 would be 975 per week	0-70° or 0 to 359° oscillation	Peoria, IL, USA
Dust Suppression Equipment	Dust Boss	DB-100	17-39 GPM	Electric	NA	1	200'	3200 pounds	68 x 119"	93	Does not have a tank	Does not have a tank	Anywhere from 50 to 200 microns	Rental of a DB-100 would be 1,600 per week	0-70° or 0 to 359° oscillation	Peoria, IL, USA
	Dust Boss	DB-60	12-26.7 gal/min. 0.5 GPM fluid flow at 55 psi air pressure		somewhere around 30 inches	1	328'	4,500 pounds	106 x 238"	101	Does not have a tank	Does not have a tank	Anywhere from 15 to 30 microns	Rental of an OB-60G would be 1,600 per week	0-70° or 0 to 359° oscillation	Peoria, IL, USA
	Major	3100LGP	varies	РТО	NA	NA	NA	19041 Kg	104 x 326"	132"	14093 Litres	Stainless Steel 3100 Gal.	NA	Varies	NA	Ballyhaunis, Co. Mayo Ireland
	Chief Tain	CVT 11500	varies	РТО	NA	NA	NA	4400kg	102 x 266"	113"	11500 Litres	Varies	NA	Varies	NA	Ballyhaunis, Co. Mayo Ireland
Foggers	Dyndafog (may not be suitable for all chemicals)	1200	120 gal/hr	engine	NA	various	NA	467 to 620 lbs	43 x 60"	42"	55 gal	NA	10-100	17,000.00	thermal fogger, but can order optional cold fog kit	Houston, TX, USA
	VectorFog	TU100	60-90 LPH	engine	NA	NA	NA	1.5 KG	35 x 51"	36"	40 gal	stainless steel	NA	19,880.00	cold fogger	Miami, FL, USA
De-Icing	Dultmeir	DU1A045	yes, varies	hydraulic pump or engine	NA	NA	~10' floor only	2500 lbs	83 x 182"	73"	NA	1800 gal polly	NA	14,978.00	flooding nozzles	Omaha, NE, USA
Equipment	Central Equipment LLC	1300 Skidded Sprayer	yes, 200 gal/min max	engine	NA	NA	~10' floor only	1100 lbs dry 15000 lbs wet	68 x 138"	75"	45 HP	1300 gal high density poly	NA	8,549.00	all wetted parts are plastic 90" spray bar	Port Byron, NY, USA

Table C-2. Commercial Equipment for Subway Decontamination (Continued)

Appendix D Mod 5 Add-on Test Results

Efficacy Results

The detailed decontamination efficacy results for 5 additional tests funded through contract modification 5 of pH-amended bleach (pAB) and 2% diluted bleach sprayed with AOF sprayer against *Bacillus atrophaeus* (*B.g.*) on two material types are shown in Table D-1. Colony forming units (CFU) were observed on all procedural blanks indicating mechanical removal and redeposition of spores.

Test											
Number								Positive Control (unsprayed)	Positive Control ^b (water sprayed)	Test Coupon ^c (Decon sprayed)	
						Ceramic Tile L1		$6.39 \pm 0.74 \text{ x } 10^7$	$6.41 \pm 0.80 \; x \; 10^7$	$3.68 \pm 2.05 \ x \ 10^7$	0.35 ± 0.37
M5-1	pAB	AOF	10	0.5	10	Ceramic Tile L2	9.10E+07	$0.39 \pm 0.74 \times 10$	$6.49 \pm 1.04 \; x \; 10^7$	0.00 ± 0.00	${\geq}7.81\pm0.07$
WI3-1	рав	AOF	10	0.5	10	Unpainted Concrete L1	9.10E+07	$1.45 \pm 0.42 \ge 10^{6}$	$2.01 \pm 1.45 \; x \; 10^6$	$2.71 \pm 1.11 \ x \ 10^5$	0.82 ± 0.33
						Unpainted Concrete L2		$1.45 \pm 0.42 \times 10^{-1}$	$3.29 \pm 2.62 \; x \; 10^6$	$3.71 \pm 2.88 \; x \; 10^4$	1.94 ± 0.40
						Ceramic Tile L1		$7.75 \pm 2.05 \text{ x } 10^7$	$6.65 \pm 1.00 \; x \; 10^7$	$8.35 \pm 1.79 \; x \; 10^4$	3.99 ± 0.94
M5-2	5-2 pAB AOF 5 0.5	0.5	10	Ceramic Tile L2	9.53E+07	$7.75 \pm 2.05 \times 10^{-10}$	$7.85 \pm 2.86 \; x \; 10^7$	0.00 ± 0.00	${\geq}7.88\pm0.12$		
N13-2	рав	AB AOF 5 0.5	10	Unpainted Concrete L1		$5.51 \pm 8.21 \ge 10^6$	$1.51 \pm 0.45 \; x \; 10^6$	$8.95 \pm 4.76 \; x \; 10^4$	1.35 ± 0.46		
						Unpainted Concrete L2		5.51 ± 8.21 x 10	$2.10 \pm 1.38 \; x \; 10^6$	$7.83 \pm 2.65 \ x \ 10^3$	2.37 ± 0.30
						Ceramic Tile L1		$6.28 \pm 0.62 \ge 10^7$	$6.05 \pm 1.53 \; x \; 10^7$	0.00 ± 0.00	${\geq}7.77\pm0.09$
M5-3	2%	AOF	2.4	0.5	10	Ceramic Tile L2	9.87E+07	$0.28 \pm 0.02 \times 10$	$1.15\pm 0.62\;x\;10^{7}$	0.00 ± 0.00	${\geq}7.22\pm0.19$
M13-3	NaOCl	AOF	2.4	0.5	10	Unpainted Concrete L1	9.07L+07	$1.17 \pm 0.46 \ge 10^6$	$1.29\pm 0.43\ x\ 10^{6}$	$1.05 \pm 0.46 \; x \; 10^4$	2.11 ± 0.23
						Unpainted Concrete L2		$1.17 \pm 0.40 \times 10$	$1.99 \pm 0.82 \; x \; 10^6$	0.00 ± 0.00	${\geq}6.27\pm0.14$
M5-4	2%	AOF	2.4	0.5	10	Unpainted Concrete L1	9.17E+07	$1.07 \pm 0.35 \text{ x } 10^6$	$1.37\pm 0.46\;x\;10^{6}$	$1.66 \pm 1.02 \ x \ 10^3$	3.47 ± 1.31
M13-4	NaOCl	AOF	2.4	0.5	10	Unpainted Concrete L2	9.1714-07	$1.07 \pm 0.33 \times 10^{-1}$	$5.62\pm 3.23\ x\ 10^{6}$	0.00 ± 0.00	${\geq}6.67\pm0.27$
						Unpainted Concrete L1			$3.82\pm 0.83\ x\ 10^{6}$	$4.40 \pm 6.69 \; x \; 10^2$	4.95 ± 1.33

Table D-1. Add-on Inactivation of Bacillus atrophaeus Spores using pH-Amended and dilute 2% Bleach^a

^a Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on three individual samples and decontamination efficacy (log reduction).

^bPositive Controls = samples inoculated, not decontaminated.

^c Test Coupons = samples inoculated, decontaminated.

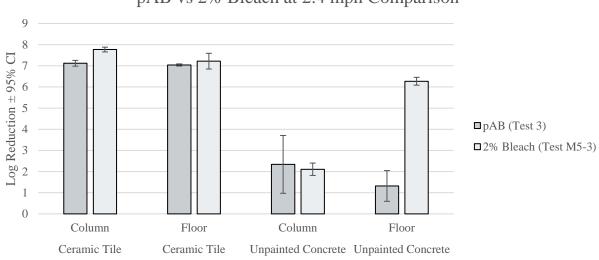
^d CI = confidence interval ($\pm 1.96 \times SE$).

^eL1 and L2 represent (L1=column, L2=floor)

Comparison of pH Amended and 2% Dilute Bleach

The decontamination efficacy of pH Amended (pAB) and two percent diluted bleach delivered via the AOF orchard sprayer against *B.g.* was evaluated on ceramic tile and unpainted concrete at two locations, contact times of 30 min, delivery speeds of 2.4 mph, and repeat applications of 1, 2 and 3 with a 30 min contact time between applications. A target temperature of 10 °C was used to represent the ambient environmental conditions that would be expected in underground subway platforms and tunnels.

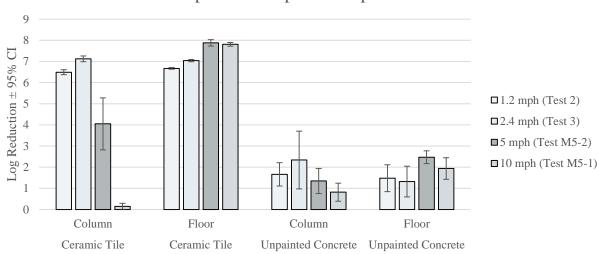
Results are organized by test condition in Figures D1 through D4 to visualize the effect of decontaminate, operational speed, and repeat application respectively. Figure E1 indicates a significant difference with two percent dilute bleach resulting in a higher log reduction (LR) for concrete at the floor location.



pAB vs 2% Bleach at 2.4 mph Comparison

Figure D1. pH amended bleach (pAB) vs 2% dilute bleach at 2.4 mph.

Similarly, as shown in Figure D2, little difference is seen between spray delivery speeds of 1.2 and 2.4 mph however when increasing speed to 5 and 10 mph a reduction of LR is observed at the column location while floor location remains similar. This finding is further supported by measurement of mass deposition of water as shown in Figure D4. At speeds of 1.2 and 2.4 mass deposition of water remains consistent, however with increased speeds of 5 and 10 mph a reduced deposition of liquid was observed.



Operational Speed Comparison

Figure D2. Efficacy of pH amended bleach (pAB) at varied application speeds.

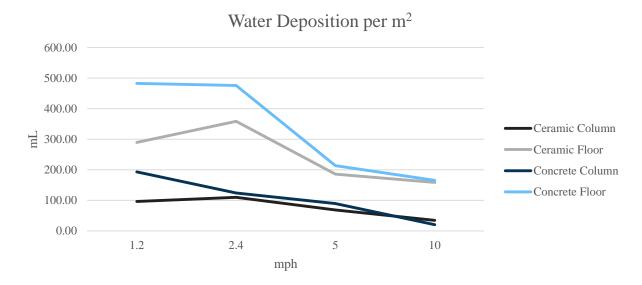


Figure D3. Mass deposition of water at various delivery speeds.

Finally, the repeat application (1, 2, and 3 applications) of two percent bleach with 30 minute contact times between each application resulted in similar LR when compared to pAB at the column location as seen in Figure D4. However, at the floor location, a significant increase in LR was noted using two percent bleach resulting in complete inactivation after just one application.

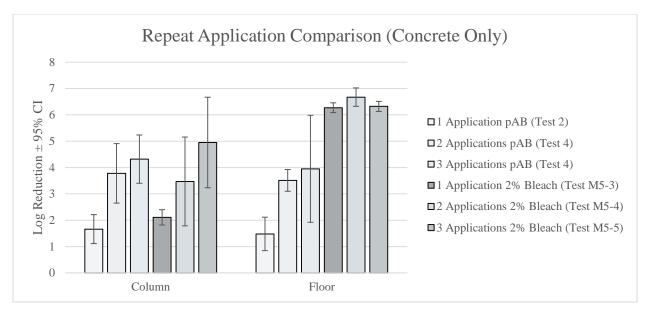


Figure D4. Efficacy of multiple applications of pH amended bleach (pAB) and 2% bleach.

Appendix E Technology Demonstration Feedback Results

Underground Transport Restoration (UTR) Operational Technology Demonstration Observer Form

DATE: (3 0CT 2016	
NAME: Roberts, Ryan	
ORGANIZATION JIF-CS	
ROLE IN THE ORGANIZATION (BRN Planner

Organizational Feedback (if you've already completed this section on another day, please skip)

1. What responsibilities does your organization have in the event of a biological contamination event? Federal response dealing with tiple 10 DaD.

2. What response capabilities does your organization currently have (e.g. decontamination technologies, survey instrumentation, etc.)

- 3. Does your organization have a detailed response plan in the case of a wide-area decontamination event?
- 4. What are the most important factors in considering purchase of and planning for use of wide area decontamination technologies? $\int f f f$

Effectiveness, efficiency, & cost

- 5. Does your organization stockpile decontamination technologies for a large contamination event? Why or why not? I dur'r Know the extent. Some, burnor enough,
- 6. Describe any current situations your organization is facing requiring biological mitigation and response? What technologies are you considering?
- 7. Please describe any other aspects of your organization you'd like us to know about.
- 8. Would you like us to contact you for detailed discussion?

Technology Demonstration Feedback

Demonstrated Technology Air-O-Fan

1. What are the observed benefits of this technology?

2. What are the observed limitations?

- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more useful? (anera, speedunery (nor (fl), remove controlled)

- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?

- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Demonstrated Technology Dust Boss

1. What are the observed benefits of this technology?

Less nu pure, nure efficient.

2. What are the observed limitations?

- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more useful?

Canva, speedonerer ... Spray below & directly sideways

- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?

- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Underground Transport Restoration (UTR) Operational Technology Demonstration Observer Form

DATE: 13 Oct 20	16					
NAME: Marwy	Grant	(Major)	VJAF			
ORGANIZATION	JTF-CS					
ROLE IN THE ORGA	NIZATION	B10	CBRN	Planner	Lead	

Organizational Feedback (if you've already completed this section on another day, please skip)

- 1. What responsibilities does your organization have in the event of a biological contamination event? Provide C2 option, trained response and detection apabilities, military and augment support.
- 2. What response capabilities does your organization currently have (e.g. decontamination technologies, survey instrumentation, etc.)

- 3. Does your organization have a detailed response plan in the case of a wide-area decontamination event?
 Yes, but hor result.
- 4. What are the most important factors in considering purchase of and planning for use of wide area decontamination technologies?

- Effective, practicability pase of use - Mubility, coverase", time "

5. Does your organization stockpile decontamination technologies for a large contamination event? Why or why not?

 6. Describe any current situations your organization is facing requiring biological mitigation and response? What technologies are you considering?

- SUBJURT Lead Fed Agency.

7. Please describe any other aspects of your organization you'd like us to know about.

- support (Lucal) training opportunitie,

8. Would you like us to contact you for detailed discussion?

Technology Demonstration Feedback

Demonstrated Technology Air-O-Fan

1. What are the observed benefits of this technology?

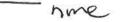
2. What are the observed limitations?

- Need a couple of run thoroushs.

3. Why or why not would this technology be helpful to your organization?

No, but WILL limit time used for decon operators

4. What additional tools/support would you need to implement use of this technology?



5. What improvements could you envision making to this technology to make it more useful?

- Vider monitors

6. Why or why not would you consider stockpiling this technology for response situations?

training requirements may be a limitah

7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?

No

- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Demonstrated Technology Dust Boss

- 1. What are the observed benefits of this technology?
- 2. What are the observed limitations?
- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more useful?
- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?
- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Underground Transport Restoration (UTR) Operational Technology Demonstration Observer Form

DATE: 13 DEC 2016	
NAME: MAS THEODORE WILSON	
ORGANIZATION JTF-CS	
ROLE IN THE ORGANIZATION FORCE	HEALTH PROTECTION

Organizational Feedback (if you've already completed this section on another day, please skip)

1. What responsibilities does your organization have in the event of a biological contamination event?

WE PREVIDE COMMAND AND CONTROL OF DOD ASSETS.

2. What response capabilities does your organization currently have (e.g. decontamination technologies, survey instrumentation, etc.)

COMMAND & CONTROL

3. Does your organization have a detailed response plan in the case of a wide-area decontamination event?

YES

4. What are the most important factors in considering purchase of and planning for use of wide area decontamination technologies?



- 5. Does your organization stockpile decontamination technologies for a large contamination event? Why or why not?
- 6. Describe any current situations your organization is facing requiring biological mitigation and response? What technologies are you considering?
- 7. Please describe any other aspects of your organization you'd like us to know about.
- 8. Would you like us to contact you for detailed discussion?

Technology Demonstration Feedback

Demonstrated Technology Air-O-Fan

- 1. What are the observed benefits of this technology? REDUCED LABCE , EASE OF USE
- 2. What are the observed limitations?

BASK SIDE & BELOW FLATBED

3. Why or why not would this technology be helpful to your organization?

REDUCES THE AMOUNT OF MAN POWER NEEDED

4. What additional tools/support would you need to implement use of this technology?

TRAINING

5. What improvements could you envision making to this technology to make it more useful?

MODIFY W/ DACKSIDE + BELOW FLAT BED SPRAYING

- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?

THE CURPLENT PLAN IS TO CONTAIN

- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Demonstrated Technology Dust Boss

1. What are the observed benefits of this technology?

REDUCTION IN MANFEWER

2. What are the observed limitations?

SPRAY COVERAGE - OSCILATION DOUSN'T COVER AS WELL

3. Why or why not would this technology be helpful to your organization?



4. What additional tools/support would you need to implement use of this technology?

TRAINING

5. What improvements could you envision making to this technology to make it more useful?



- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?

CURRENT PLAN IS TO CONTAIN

- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Underground Transport Restoration (UTR) Operational Technology Demonstration Observer Form

DATE: 13 Oct 16 NAME: Chris Coupy ORGANIZATION 7th SFG(A) / 26th CRD ROLE IN THE ORGANIZATION CBRN NLO

Organizational Feedback (if you've already completed this section on another day, please skip)

- 1. What responsibilities does your organization have in the event of a biological contamination event? Our responsibility would be to recognize a hazard, and potentily sample if aquited propper rescurses.
- 2. What response capabilities does your organization currently have (e.g. decontamination technologies, survey instrumentation, etc.) We have most of the Suifs the epa uses Level A D, quick silver sampling kits, HHAS.
- 3. Does your organization have a detailed response plan in the case of a wide-area decontamination event? No typically we operate in small man teams of 3-4.
- 4. What are the most important factors in considering purchase of and planning for use of wide area decontamination technologies?
- 5. Does your organization stockpile decontamination technologies for a large contamination event? Why or why not?
- 6. Describe any current situations your organization is facing requiring biological mitigation and response? What technologies are you considering?
- Please describe any other aspects of your organization you'd like us to know about.
 We are currently trying to adapt and mold our Sop's on decan and sampling of bio agents any thing that could help us would be grat
 Would you like us to contact you for detailed discussion?

I would greatly appreciate your sops, and any info that you could thraw your our way to help us adapt our sop

Technology Demonstration Feedback

Demonstrated Technology Air-O-Fan

- 1. What are the observed benefits of this technology? Definitely covered the entire tunnel with water at least ceilings & walls
- 2. What are the observed limitations? (ould adapt a system to focus more on the grand
- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more useful?

Mayloe adding a few sports tocused tawards the grand level

6. Why or why not would you consider stockpiling this technology for response situations?

- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?
- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Demonstrated Technology Dust Boss

- 1. What are the observed benefits of this technology? Great for potentially spraying one area
- 2. What are the observed limitations?
- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more useful? This could be great with a faster swirel at a lover speed.
- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?
- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Underground Transport Restoration (UTR)
Operational Technology Demonstration Observer Form
DATE: 10 13 2016
NAME: Georgeann Morekas
ORGANIZATION CSS-Bynamac
ROLE IN THE ORGANIZATION Diverter there use Recovery Busevis Fine

Organizational Feedback (if you've already completed this section on another day, please skip)

- 1. What responsibilities does your organization have in the event of a biological contamination event?
- 2. What response capabilities does your organization currently have (e.g. decontamination technologies, survey instrumentation, etc.)
- 3. Does your organization have a detailed response plan in the case of a wide-area decontamination event?
- 4. What are the most important factors in considering purchase of and planning for use of wide area decontamination technologies?
- 5. Does your organization stockpile decontamination technologies for a large contamination event? Why or why not?
- 6. Describe any current situations your organization is facing requiring biological mitigation and response? What technologies are you considering?
- 7. Please describe any other aspects of your organization you'd like us to know about.
- 8. Would you like us to contact you for detailed discussion?

Technology Demonstration Feedback

Demonstrated Technology Air-O-Fan

1. What are the observed benefits of this technology? Remote operation; Commercially available; significant coverage good ceiling covering What are the observed limitations? No Cover behind poles, structures, etc.

- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more

useful? Suggest combining the two units for complete livenge. 1.5ft. Wele/ceiler were not exposed to solution 6. Why or why not would you consider stockpiling this technology for response situations?

- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?
- Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Demonstrated Technology Dust Boss

1. What are the observed benefits of this technology?

Remote operation. Commercially available - abolity to adjust full of some -2. What are the observed limitations? Improved but not complete coverage bellend pole structures etc.

- 3. Why or why not would this technology be helpful to your organization?
- 4. What additional tools/support would you need to implement use of this technology?

5. What improvements could you envision making to this technology to make it more useful? Allower and upper Spray Fredd

- 6. Why or why not would you consider stockpiling this technology for response situations?
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?
- 8. Please provide any other comments
- 9. Would you like us to contact you for detailed discussion?

Underground Transport Restoration (UTR) Operational Technology Demonstration Observer Form DATE: 131 Philip J Frank NAME: ORGANIZATION 7th Group SFG (A) Army ROLE IN THE ORGANIZATION CBRN Specialist

Organizational Feedback (if you've already completed this section on another day, please skip)

- 1. What responsibilities does your organization have in the event of a biological contamination event? Take samples / Decon line if Oconus
- 2. What response capabilities does your organization currently have (e.g. decontamination technologies, survey instrumentation, etc.) MOPP Goer / cvel A, BC, & D SC, B.A. Dr. SKO3 K:13
- Does your organization have a detailed response plan in the case of a wide-area decontamination event?
- 4. What are the most important factors in considering purchase of and planning for use of wide area decontamination technologies? Cost, is it more efficient then man power, if it is safe.
- 5. Does your organization stockpile decontamination technologies for a large contamination event? Why or why not? Yes, we need to be able to decon large amounts of bodies /vehicles.
- Describe any current situations your organization is facing requiring biological mitigation and response? What technologies are you considering?
 Actions in cw AO cversecs.

7. Please describe any other aspects of your organization you'd like us to know about.

8. Would you like us to contact you for detailed discussion?

Technology Demonstration Feedback

Demonstrated Technology Air-O-Fan

- 1. What are the observed benefits of this technology? Keeping people Safe, net abt of chances for people to become conteminated
 2. What are the observed limitations?, Cost efficient, would need more tanks of water.
- 3. Why or why not would this technology be helpful to your organization? Our HO is most oversees iso cost of buying I shipping would be great.
- 4. What additional tools/support would you need to implement use of this technology?
- 5. What improvements could you envision making to this technology to make it more useful? more then one being used and more tanks for water. Metel hosses so they don't deferre
- 6. Why or why not would you consider stockpiling this technology for response situations? Incase of a wide spread bio attack.
- 7. Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste? CS.
- 8. Please provide any other comments n/A
- 9. Would you like us to contact you for detailed discussion?

Demonstrated Technology Dust Boss

- 1. What are the observed benefits of this technology?
- 2. What are the observed limitations? Speec of osoletions, vor: chies of osoletions, vor: chies of other pressure.
- 3. Why or why not would this technology be helpful to your organization? Shipping Cost/budget
- 4. What additional tools/support would you need to implement use of this technology? People to set if up I classes on hew to run if.
- 5. What improvements could you envision making to this technology to make it more useful? Heving one of more working at once.
- 6. Why or why not would you consider stockpiling this technology for response situations? We wouldn't dear such a large contamined area in our AG.
- Upon generation of secondary aqueous waste, does your organization generally plan or prefer to contain or treat the waste?
- 8. Please provide any other comments

1/14

9. Would you like us to contact you for detailed discussion?



Office of Research and Development (8101R) Washington, DC 20460

Official Business Penalty for Private Use \$300 PRESORTED STANDARD POSTAGE & FEES PAID EPA PERMIT NO. G-35