Evaluation of Liquid and Foam Decontamination Technologies for Surfaces Contaminated by *Bacillus anthracis* Spores

EPA investigates the effectiveness of liquid and foam decontamination technologies for surfaces contaminated with biological agents

Background

Because of their potential use as weapons of mass destruction, biological agents are a significant terrorist threat. Once released, agents such as bacteria and viruses can cause disease or death in humans, animals, and plants by spreading through air, water distribution systems, and the food supply.

Bacillus anthracis—the spore-forming bacterium which causes anthrax—is one of the most likely biological agents to be used by terrorists. In the United States, twenty-three people became infected with anthrax and five died after envelopes containing *B. anthracis* spores were mailed to governmental and news media offices during the months following the Sept. 11 terrorist attacks. Sites where letters were received and many U.S. postal service facilities became contaminated with spores.

As part of U. S. EPA's Office of Research and Development, the National Homeland Security Research Center (NHSRC) provides products and expertise to improve our nation's ability to respond to environmental contamination caused by terrorist attacks on our nation's water infrastructure, buildings and outdoor areas.

NHSRC conducts research related to:

- Detecting and containing contamination from chemical, biological, and radiological agents
- Assessing and mitigating exposure to contamination
- Understanding the health effects of contamination
- Developing risk-based exposure advisories
- Decontaminating and disposing of contaminated materials.

Although person to person transmission has not been demonstrated, humans can acquire anthrax by contact with spores. Anthrax is a naturally occurring disease most commonly found in grazing animals such sheep, cattle, and goats. Spores can be found in the tissues from infected animals or in contaminated products made from bone, hide, wool, or hair.

Spores pose a continuing threat because they are viable for decades, even under extreme environmental conditions. *B. anthracis* spores can be processed or weaponized and delivered through the air over wide areas. A major attack using *B. anthracis* spores could cause many deaths and interrupt vital civilian and government operations.

One of the key challenges following an anthrax attack is cleaning up contaminated areas for re-entry and re-use. The primary goal is to reduce the cost and time it takes to remediate an area while protecting workers and nearby residents.

The challenge: find decontaminant technologies that are effective against spores

B. anthracis forms spores that are highly resistant to severe environmental conditions, including exposure to harsh chemicals. In 2001, when remediation of facilities contaminated by *B. anthracis* spores began, there were no EPA-registered products specifically for use against the spores. EPA's Office of Pesticide Programs had to issue crisis exemptions for the sporicidal products needed for remediation.

April 2011

The Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel[1] was convened in 2007 to provide guidance on test methods for determining on the efficacy of antimicrobial products for inactivating *B. anthracis* spores. The Panel proposed that, in order to be registered as a sporicidal decontaminant against *B. anthracis* spores, a decontaminant technology had to achieve a mean (average) 6 $\log_{10}$ reduction in the number of viable spores.

EPA's decontamination technology evaluation research

EPA conducted tests to collect performance (efficacy) data on a variety of products and technologies that might be able to decontaminate surfaces contaminated with *B. anthracis* spores [1, 2, 3]. Decontamination technologies were investigated under conditions similar to those likely to occur in buildings or outdoor populated areas. Although one of the major factors influencing the decontaminant effectiveness is the type of material being decontaminated, a number of other issues are important, as shown in Table 1.

Table 1 Factors That Influence Decontaminant Effectiveness

• Relative humidity
• Temperature
• Characteristics and amount of the biological agent on the surface
• Type of material or porosity of a surface being decontaminated
• How long the decontaminant is in contact with the surface or material
• Concentration of the decontaminant

Table 2 lists general descriptions of the twelve decontaminants tested.

Table 2 Liquid and Foam Decontaminant Technologies Tested

Decontamination Technology	Description/Active Ingredients	Vendor/Source	Abbreviations Used in Tables 4,5,6
Calcium polysulfide	Calcium polysulfide	VGS, Inc.	Cal poly
CASCAD™ Surface Decontamination Foam (SDF)	Hypochlorite, hypochlorous acid	Allen-Vanguard Corp.	CASCAD
Decon Green	Hydrogen peroxide	Developed by the U.S. Army	Decon Green
DioxiGuard™	Chlorine dioxide	Frontier Pharmaceutical Inc.	DioxiGuard
EasyDECON® 200	Hydrogen peroxide	EFT Holdings Inc.	EasyDECON
Klozur™	Sodium persulfate, hydrogen peroxide	FMC Corp.	Klozur
MINNCARE® Cold Sterilant	Hydrogen peroxide, peracetic acid	Minntech Corp.	MINN
Oxonia Active®	Hydrogen peroxide, peracetic acid	Ecolab Inc.	Oxonia
Peridox® RTU	Hydrogen peroxide, peracetic acid	CET LLC	Peridox
SanDes	Chlorine dioxide	DTI-Sweden AB	SanDes
Spor-Klenz® RTU	Hydrogen peroxide, peracetic acid	STERIS Corp.	Spor-Klenz
Ultra Clorox® Germicidal Bleach [a]	Sodium hypochlorite, hypochlorous acid	The Clorox Co.	pH Bleach

[a] Bleach was amended by diluting with water and using acetic acid to lower the pH to between 6 and 7

Tests were conducted using the decontaminants on one or more of eighteen materials. Glass and topsoil were test materials in two studies. In each technology evaluation, *B. anthracis*

[1] Final Meeting Minutes for July 17 – 18, 2007 Scientific Advisory Panel: Guidance on Test Methods for Demonstrating the Efficacy of Antimicrobial Products for Inactivating Bacillus anthracis Spores on Environmental Surfaces

spores were spiked on coupons made from representative porous or non-porous materials used in buildings or outdoors.

As seen in Table 3, eight decontaminant technologies achieved higher than mean 6 $\log_{10}$ reductions of viable *B. anthracis* spores on at least five materials. Seven achieved complete spore inactivation on five or more materials.

CASCAD™ SDF, Decon Green, EasyDECON® 200, MINNCARE® Cold Sterilant, Oxonia Active®, and Peridox® RTU inactivated spores on 70% or more of materials tested.

Table 3 Decontaminants Tested, Number of Materials Showing Mean 6 Log_{10} Spore Reductions or Higher, and Contact Times

Decontaminant Technology	Number of Materials Tested	Number of Materials on Which a Higher Than Mean 6 Log_{10} Reductions in Spores Was Observed	Number of Materials on Which Spores Were Inactivated Completely	Contact Times of Decontaminants on Materials
Calcium polysulfide	4	0	0	60 min
CASCAD™ SDF	17	14	12	30 min 120 min (topsoil)
Decon Green	10	7	7	60 min
DioxiGuard™	7	0	0	10 min
EasyDECON® 200	10	8	8	30 min (non-porous) 60 min (porous)
Klozur™	1	0	0	48 hours (topsoil)
MINNCARE® Cold Sterilant	7	6	6	10 min (non-porous) 30 min (porous)
Oxonia Active®	8	6	5	60 min
Peridox® RTU	10	8	7	30 min (non-porous) 60 min (porous)
SanDes	7	0	0	70 min
Spor-Klenz® RTU	10	8	4	30 min (non-porous) 60 min (porous)
Ultra Clorox® Germicidal Bleach [a]	14	9	7	60 min

[a] Bleach was amended by diluting with water and using acetic acid to lower the pH to between 6 and 7

Only two of the twelve decontaminants caused any visible damage to the materials being decontaminated. Calcium polysulfide left grayish residue on glass and topsoil coupons. The residue was not removed from the glass during any spore extraction processing for quantitative or qualitative analysis. Because of material surface characteristics, it could not be determined whether the residue was also left on the bare pine wood or unpainted concrete. CASCAD™ SDF on painted cinder block coupons caused the top coat of paint to peel away from the primer coat.

Tables 4 and 5 present the results of the decontaminant technology evaluations. Generally, more of the decontaminants achieved higher mean $\log_{10}$ reductions in the number of viable spores on non-porous materials than on porous materials. However, some decontaminants achieved greater than mean 6 $\log_{10}$ reductions on both types of materials.

Table 4 Summary Results on the Efficacy of Liquid and Foam Decontaminants on Non-porous Materials Contaminated With *Bacillus anthracis* Spores

Materials Tested	Ranges of Mean Log_{10} Reductions						
	> 6	5 to 6	4 to 5	3 to 4	2 to 3	1 to 2	0 to 1
Aluminum [1]	※[a] CASCAD [b] ※ Decon Green ※ EasyDECON ※ Peridox ※ pH Bleach [c] Spor-Klenz						
Decorative Laminate [2]	CASCAD ※ MINN ※ Oxonia				DioxiGuard		SanDes
Galvanized Metal Ductwork [2]	※ CASCAD ※ MINN ※ Oxonia						DioxiGuard SanDes
Glass [1,2]	※ CASCAD [1,2] ※ Decon Green [1] ※ EasyDECON [1] ※ MINN [2] ※ Oxonia [2] ※ Peridox [1] ※ pH Bleach [1,2] Spor-Klenz [1]		SanDes [2]		DioxiGuard [2]		Cal poly [2]
Glazed Porcelain [1]	※ CASCAD ※ Decon Green ※ EasyDECON ※ Peridox ※ pH Bleach ※ Spor-Klenz						
Sealed Granite [1]	※ CASCAD ※ Decon Green ※ EasyDECON ※ Peridox ※ pH Bleach ※ Spor-Klenz						
Stainless Steel [1]	※ CASCAD ※ Decon Green ※ EasyDECON ※ Peridox ※ pH Bleach Spor-Klenz						

[a] ※ No colony forming units were found in the extracts from the materials following decontamination
[b] See Table 3 for contact times
[c] Bleach was amended by diluting with water and using acetic acid to lower the pH to between 6 and 7

Table 5 Summary Results on the Efficacy of Liquid and Foam Decontaminants on Porous Materials Contaminated with *Bacillus anthracis* Spores

Materials Tested	Ranges of Mean Log_{10} Reductions						
	> 6	5 to 6	4 to 5	3 to 4	2 to 3	1 to 2	0 to 1
Asphalt Paving Aggregate (Fine) [1]	※[a] CASCAD [b] Peridox			pH Bleach [c]	Decon Green Spor-Klenz	EasyDECON	
Bare Pine Wood [2]		MINN	Oxonia		CASCAD		Cal poly DioxiGuard pH Bleach SanDes
Butyl Rubber Sealant Tape [1]	※ CASCAD ※ Decon Green ※ EasyDECON ※ Peridox ※ pH Bleach ※ Spor-Klenz						
Concrete [1]	※ CASCAD ※ EasyDECON pH Bleach		Decon Green			Peridox Spor-Klenz	
Industrial Grade Carpet [2]	CASCAD ※ MINN Oxonia					DioxiGuard	SanDes
Painted Cinder Block [2]	※ CASCAD ※ MINN ※ Oxonia pH Bleach					DioxiGuard	SanDes
Painted Wallboard Paper [2]	※ MINN ※ Oxonia		CASCAD				DioxiGuard SanDes
Paving Brick [1]	※ CASCAD ※ Decon Green ※ EasyDECON ※ pH Bleach ※ Spor-Klenz			Peridox			
Topsoil [2,3]				Klozur [d] [3]		CASCAD [e] [3] Oxonia [3] pH Bleach [2]	Cal poly [2] pH Bleach [3]
Treated Wood [1]	※ CASCAD ※ Peridox Spor-Klenz					Decon Green pH Bleach	EasyDECON
Unpainted Concrete [2]			pH Bleach				Cal poly

[a] ※ No colony forming units were found in the extracts from the materials following decontamination

[b] See Table 3 for contact times

[c] Bleach was amended by diluting with water and using acetic acid to lower the pH to between 6 and 7

[d] Contact time was 48 hours; the Klozur™ technology, which uses hydrogen peroxide and persulfate chemistry, was tested at relatively longer contact times based upon its typical field-use conditions and achieved a 3.50 log_{10} reduction after 48 hours of contact

[e] Contact times for CASCAD, Oxonia, and pH Bleach [3] were 120 min

B. subtilis was included in one of the investigations [2] to provide data for a non-pathogenic organism that might suffice for use in testing as a surrogate for *B. anthracis.*

Table 6 shows that test results obtained with spores from *B. subtilis* are similar, but not identical to, results obtained with *B. anthracis* spores. These results underscore that, although experimental results using surrogates might be indicative of the behavior of biological agents, they are not necessarily predictive.

Table 6 Summary Results on the Efficacy of Liquid and Foam Decontaminants on Porous and Non-porous Materials Contaminated with *Bacillus subtilis* Spores

Materials Tested	Ranges of Mean Log_{10} Reductions						
	> 6	5 to 6	4 to 5	3 to 4	2 to 3	1 to 2	0 to 1
Bare Pine Wood [2]	MINN [a]	Oxonia				CASCAD	Cal poly DioxiGuard pH Bleach [b] SanDes
Decorative Laminate [2]	※[c] CASCAD ※ MINN ※ Oxonia					SanDes	
Galvanized Metal Ductwork [2]	※ CASCAD ※ MINN ※ Oxonia						DioxiGuard SanDes
Glass [2]	※ CASCAD ※ MINN ※ Oxonia						Cal poly DioxiGuard San Des
Industrial Grade Carpet [2]	※ CASCAD ※ MINN ※ Oxonia						DioxiGuard SanDes
Painted Cinder Block [2]	※ CASCAD ※ MINN ※ Oxonia ※ pH Bleach						DioxiGuard SanDes
Painted Wallboard Paper [2]	※ CASCAD ※ MINN ※ Oxonia						DioxiGuard SanDes
Topsoil [2]							Cal poly pH Bleach
Unpainted Concrete [2]							Cal poly DioxiGuard

[a] See Table 3 for contact times

[b] Bleach was amended by diluting with water and using acetic acid to lower the pH to between 6 and 7

[c] ※ No colony forming units were found in the extracts from the material following decontamination

Technology Evaluation Reports Referenced

[1] Calfee, M.W. 2010. *Biological Agent Decontamination Technology Testing*. Technology Evaluation Report. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-10/087.

[2] Wood, J. 2009. *Evaluation of Liquid and Foam Technologies for the Decontamination of B. anthracis and B. subtilis on Building and Outdoor Materials*. Technology Evaluation Report. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-09/150.

[3] U.S. Environmental Protection Agency. 2010. *Evaluation of Liquid and Foam Technologies for the Inactivation of Bacillus anthracis Spores in Topsoil*. Investigation Report. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-10/080.

Contact Information

For more information, visit the NHSRC Web site at www.epa.gov/nhsrc.

Technical Contacts: Joseph Wood (wood.joe@epa.gov
Worth Calfee (calfee.worth@epa.gov)

General Feedback/Questions: Kathy Nickel (nickel.kathy@epa.gov)