Updates and Developments to EPA's Water Contaminant Information Tool (WCIT)

John Bain, ORISE Participant, and George Gardenier, Chemist, Water Laboratory Alliance
U.S. Environmental Protection Agency, Office of Water, Water Security Division

Background

What is WCIT?

- The Water Contaminant Information Tool (WCIT) is EPA's secure, web-based informational database on priority contaminants of concern for drinking water and wastewater systems.

Information in WCIT

- There are currently over 800 CBR contaminants of concern to water systems housed in the database.

Who can use WCIT?

- WCIT access is limited to certain users, including federal employees, drinking water and wastewater utilities, public health officials and laboratories.

How can WCIT be used?

- WCIT assists users prior to, during, and after a contamination incident by providing important information on contaminants
- For more information on WCIT and upcoming training webinars visit our websites:
  - https://www.epa.gov/waterlabnetwork
  - https://www.epa.gov/waterlabnetwork/access-water-contaminant-information-tool

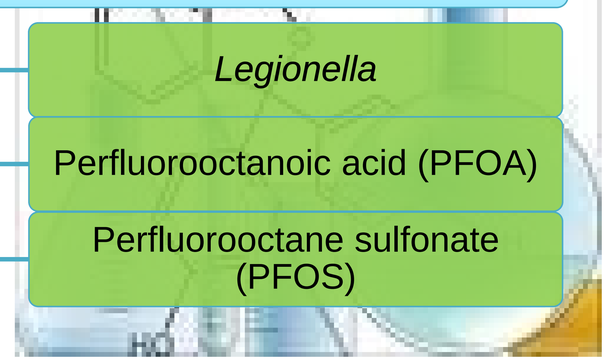
Recent & Upcoming Updates

The Water Contaminant Information Tool (WCIT) is a living tool – constantly being updated

Recently Added:

- *Microcystins & cylindrospermopsin*
- *Listeria monocyotogens*
- *Naegleria fowleri*

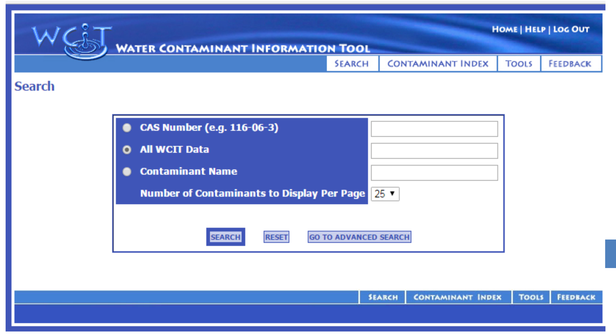
In Progress:



- *Legionella*
- Perfluorooctanoic acid (PFOA)
- Perfluorooctane sulfonate (PFOS)

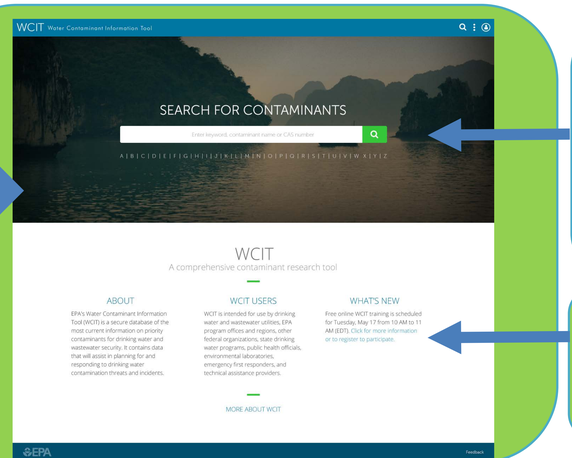
Technology Development

Current WCIT layout & search function



- Since the Water Contaminant Information Tool (WCIT) was first released technology has advanced significantly.
- Working on developing a mobile website.

Example of possible new layout & search function



Condensed and improved search function

Updated look for the homepage

Exposure and Pathways Analysis of Infectious Livestock Carcass Management Options during Emergency Situations

Sandip Chattopadhyay[1], Joshua Cleland[2], Margaret McVey[2], Kaedra Jones[2], Paul Lemieux[1], Sarah Taft[1], Lori Miller[3]
[1]U.S. Environmental Protection Agency, Office of Research and Development; [2]ICF; [3]U.S. Department of Agriculture-Animal and Plant Health Inspection Service

United States Environmental Protection Agency

Background

Management of livestock carcasses following large-scale mortalities is needed to protect humans, livestock, and wildlife from hazards; to maintain water, air, and soil resources; to protect ecological resources; and to enhance food and agricultural security. Previous health and environmental assessments (e.g., CAST 2009; Gwyther et al. 2011; NABCC 2004; Pollard et al. 2008; UKDH 2001) of mass livestock mortality events relied on qualitative evaluation of exposure or observations based on incident-specific circumstances, which limits their usefulness for general decision-making.

To address the need for a quantitative analysis, the U.S. Department of Homeland Security, U.S. Department of Agriculture, and U.S. Environmental Protection Agency are jointly conducting an exposure assessment for potential releases of chemical toxicants and microbes of seven livestock carcass management options (Table 1) and associated carcass handling and transportation activities.

Exposure estimates have ranked for the livestock carcass management options for a hypothetical site. Four mortality scenarios are being evaluated: natural disaster, foot-and-mouth disease (FMD) outbreak, a chemical contamination incident and a radiological contamination incident. This presentation focuses on the natural disaster emergency and human exposure to microbes.

Problem Formulation

The scope of the assessment includes the disaster scenario, scale of mortality, chemical and microbial hazards, and management options assessed. The management options considered for the exposure assessment are shown in Table 1.

To focus the assessment on differences among the carcass management options, exposures are assessed for each option using a hypothetical site setting and mortality scenarios. 50 U.S. tons (45,359 kg) of carcasses are considered for all management options. Exposure receptors include adult and child residents of the hypothetical farm and workers engaged in carcass management. Potential exposure pathways include inhalation, ingestion of drinking water, and ingestion of homegrown foods. Home grown foods include fruits and vegetables; livestock products including beef, dairy, pork, poultry, and eggs; and fish caught in an on-site lake. Drinking water for the farm family is obtained from an on-site well.

Table 1. Livestock Carcass Management Options for the Exposure Assessment.

Livestock Carcass Management Options
Combustion-based Management
· **On-site Open Burning --** Burning of carcasses in combustible heaps called pyres. It requires combustible material additives (e.g., straw, hay, coal) to reach necessary temperatures to completely consume animal carcasses. Pyres are layered with combustible material underneath the carcasses with space for sufficient air circulation.
· **On-site Air-Curtain Burning --** Carcasses are burned in a partially enclosed (partially open on top) refractory-lined firebox. A forced air flow, driven by a diesel-powered blower, creates a recirculation zone over the burn area that retains much of the smoke and soot within the fire box. This process allows higher temperatures to be reached and more complete combustion than open pyre burning.
· **Off-site Fixed-facility Incineration --** Commercial scale incinerators are usually fueled with propane, diesel, or natural gas, and burn solid waste through a series of stages. To be used for carcass disposal, an incinerator must be able to handle high moisture content (~70%) and long burn times to allow complete combustion. Most municipal waste-to-energy incinerators do not accept carcasses for disposal.
Land-based Management
· **On-site Unlined Burial --** Carcasses are placed in an unlined trench or a lined pit. After placement, carcasses are covered with at least 6 feet of soil, including 3 feet of soil mounded above ground level.
· **On-site Composting --** Carcass disposal via composting is highly controlled, managed, and regulated. Additional materials are required such as carbon sources, bulking agents, and biofilters (layer of carbon source and bulking material enhances microbial activity by regulating moisture content, pH, temperature, etc.).
· **Off-site Lined Landfill --** Landfills are engineered structures where waste is isolated form the surrounding environment. Some municipal solid waste landfills (RCRA Subtitle D) are specifically designated for livestock carcass disposal. Landfills must include composite liners and clay to protect groundwater and soil from the leachate, and they must implement leachate collection and removal systems.
Materials Processing
· **Off-site Rendering --** Rendering occurs in regulated facilities and transforms animal carcasses (including whole animals, carcass trimmings, inedible offal and bones) into three distinct end-products: carcass meal, melted fat/tallow, and water.

Hazard Identification

Hazardous chemicals and microbes of concern that are released directly from decomposing carcasses or from carcass management (including combustion products and added materials) and post-management processes (e.g., compost application, ash burial) were identified. Microbes of concern were identified as those that could be present in healthy cattle in the United States. Table 2 identifies the scope of microbial hazards from six gram-positive bacteria, seven gram-negative bacteria, three protozoa, six viruses, a fungus, and a prion. Even though some of these agents are unlikely to be present in U.S. cattle herds, they are included as worst-case situation.

Potential for microbial exposures was evaluated for each on-site option based on the likely occurrence, persistence, and mobility. These microbes are included as potential hazards in the livestock carcass storage, transportation, and handling stage as well as for the on-site unlined burial option, because there are no initial assumptions on thermal conditions that eliminate the consideration of any microbes for these stages of carcass management. The temperatures reached during combustion-based management options and composting vary with the duration that these temperatures are reached. Therefore, heat-resistant prions that cause bovine spongiform encephalopathy (BSE) may remain viable even after exposure to the temperatures reached during on-site open burning and on-site composting. Spore-forming bacteria may also survive the composting process.

No microbial hazards are quantitatively evaluated for off-site management options, because all environmental releases from these options are subject to existing regulations that are assumed to protect human health and the environment. Although exposures are not quantified for these options, they are included in the relative ranking of options.

Table 2. Microbial Hazards Possibly Associated with Livestock Carcass Management.

Management Type and Options	Potential Microbial Hazards at Each Stage of Livestock Carcass Management	
	Livestock Management Options	**Storage, Transportation, and Handling Activities**
Combustion-based Management	**On-site open burning:** •Prions (PrP^{Sc})[a]	**Identified pathogens:** • *Bacillus anthracis*[a] • *Campylobacter* spp. • *Clostridium perfringens* • *Coxiella burnetii* • *Dermatophilus congolensis* • *Escherichia coli* O157:H7 and other shiga-toxin producing strains • *Leptospira* spp. • *Listeria monocytogenes* • *Mycobacterium avium Paratuberculosis* • *M. bovis* • *Salmonella* spp. • *Shigella* spp. • *Yersinia enterocolitica* • *Cryptosporidium* spp. • *Giardia* spp. • *Toxoplasma gondii* • *Trichophyton verrucosum* • Rotavirus • Hepatitis E virus[a] • Influenza D virus[a] • Enteroviruses • Adenoviruses • Caliciviruses (e.g., norovirus) • Prions (PrP^{Sc})[a]
	On-site air-curtain burning: •None	
	Off-site fixed-facility Incineration: •None	
Land-based Management	**On-site unlined burial:** •All identified microbes (listed at right)	
	On-site composting: •*B. anthracis*[a]; •*C. perfringens;* •*Coxiella burnetii;* •Prions (PrP^{Sc})[a]	
	Off-site lined landfill: •None	
Rendering	• None	

[a] Microbes with a low likelihood of presence in the U.S.

Conceptual Models - Exposure Pathways

Conceptual models were developed for each of the seven livestock carcass management options and associated handling and transportation activities. Examples are presented in Figures 1 and 2 for the on-site combustion and carcass burial options, respectively.

These conceptual models were used to identify exposure pathways for microbes and chemical toxicants. Table 3 shows the exposure pathways for microbes. For most exposure pathways, potential exposures were determined to be negligible based on source conditions or properties related to survival, fate, and transport for a specific microbe. Exposure pathways determined to have potentially non-negligible exposures are in shaded cells in Table 3.

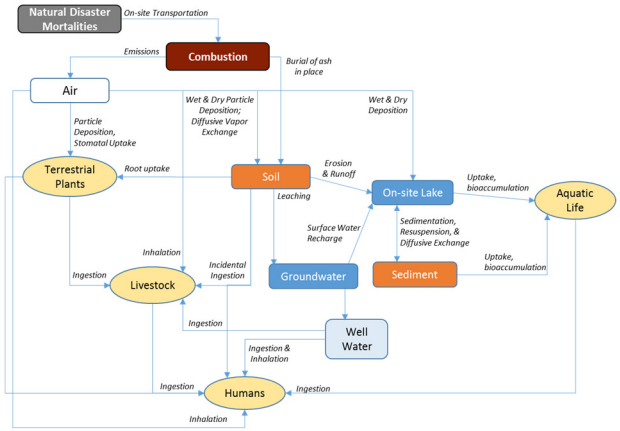


Figure 1. Conceptual Model for Open Burning and Air-curtain Burning.

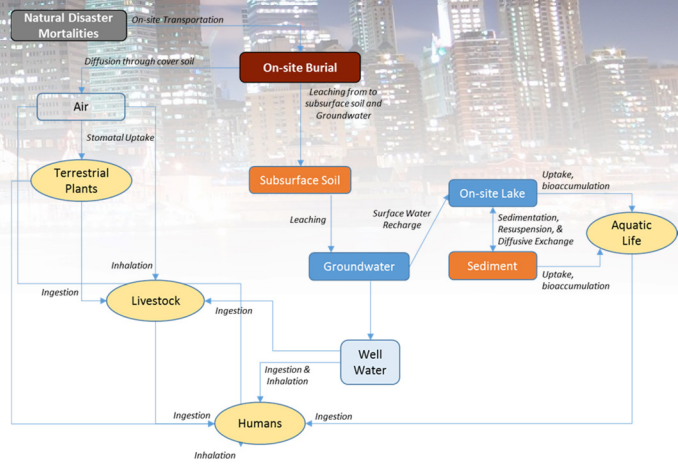


Figure 2. Conceptual Model for Carcass Burial.

Table 3. Exposure Pathways for Livestock Carcass Management Options – Microbes.

Exposure Route and Medium	Exposure Pathways – Transportation and Handling Activities			Exposure Pathways – Management Options						
	Carcass Handling	Temporary Carcass Storage	Carcass Transport-ation	Open Burning	Air-curtain Burning	Burial	Composting	Off-site Incineration	Off-site Landfilling	Rendering
Inhalation	1) Air → Inhalation[c]	1) Air → Inhalation[c] 2) Leachate → Soil → GW → Aerosol	1)Aerosol[b]	1) Air[b] 2) Ash → GW → Aerosol[b]	1) Air[b] 2) Ash → GW → Aerosol[b]	1) Air[b] 2) Leachate → GW → Aerosol[b]	1) Air[b] 2) Compost → GW → Aerosol[b]	1) Air[c]	1) Air[c]	1) Air[c]
Direct Ingestion	2) Hand-to-mouth oral contact[c]	–	–	–	–	–	–	–	–	–
Incidental Soil Ingestion	–	–	–	3) Air → Soil[b]	3) Air → Soil[b]	–	–	2) Air → Soil[c]	–	–
Fish Ingestion	–	3) Leachate → Soil → GW → SW → Fish ingestion[b]	–	4) Air → SW → Fish[b] 5) Air → soil → SW → Fish[b] 6) Ash → GW → SW → Fish[b]	4) Air → SW → Fish[b] 5) Air → Soil → SW → Fish[b] 6) Ash → GW → SW → Fish[b]	3) Leachate → GW → SW → Fish[b]	3) Compost → Soil → SW → Fish[b] 4) Compost → GW → SW → Fish[b]	3) Air → SW → Fish[c] 4) Air → Soil → SW → Fish[c]	–	–
Ground-water Ingestion	–	**4) Leachate → Soil → GW → Drinking water ingestion[a]**	–	**7) Ash → GW[a]**	7) Ash → GW[b]	**4) Leachate → GW[a]**	**5) Compost →Leachate → GW[a]**	–	–	–
Ingestion of Food Produced on the Farm	–	–	–	8) Air → Plants/ Livestock[b] 9) Air → Soil → Plants/ Livestock[b] 10) Ash → GW → Livestock[b]	8) Air → Plants/ livestock[b] 9) Air → Soil → Plants/ Livestock[b] 10) Ash → GW → Livestock[b]	5) Air → Plants/ Livestock[b] 6) Leachate → GW → Livestock[b]	6) Air → Plants/ Livestock[b] 7) Compost → Soil → GW → Livestock[b]	5) Air → Plants/ Livestock[c] 6) Air → Soil → Plants/ Livestock[c]	2) Air → Plants/ Livestock[c]	2) Air → Plants/ Livestock[c]
Dermal Contact	3) Dermal contact[c]	--	–	–	–	–	–	–	–	–

Abbreviations: "—" = no exposure pathways; SW = surface water; GW = groundwater.
Note: Exposure pathways shown in bold were included in the quantitative exposure assessment.
[a] Quantitative assessment conducted; results are presented in Section 6.2. [b] Potential exposures are assumed to be negligible based on source conditions or microbial properties.
[c] Environmental releases or exposures are assumed to be adequately controlled by existing pollution control regulations or use of personal protective equipment.

Work-in-progress

- **Quantitative analysis of exposure to FMD.** Exposures are being assessed using existing, peer-reviewed modeling tools, including EPA developed ***AERMOD*** and ***Virulo***.
- **Ranking of management options** relative to each other based on the following factors: (1) the number conceptual model pathways; (2) the number of pathways with potential microbial exposure; (3) the number of quantified pathways; and (4) quantified exposures. Numbers of conceptual model pathways and potential exposures for each option are included to account for pathways with unquantified exposure levels.
- **Exposure assessments for the chemical and radiological attacks/release incidents.**

References

CAST (Council for Agricultural Science and Technology) (2009). Issue Paper 41. Ames, Iowa.

Gwyther CL, et al. (2011). Waste Management 31(4): 767-778.

NABCC (National Agricultural Biosecurity Center Consortium) (2004). Report prepared by the NABCC, Carcass Disposal Working Group, For the USDA Animal & Plant Health Inspection Service, Per Cooperative Agreement 02-1001-0355-CA.

Pollard S, et al. (2008). Environ. Sci. Technol. 42: 3145-3154.

United Kingdom Department of Health (UKDH) (2001). A Rapid Qualitative Assessment of possible risks to Public Health from current Foot & Mouth Disposal Options.

Operational Field Demonstration

Outdoor Biological Simulant Releases in an Operationally Relevant Environment

Amanda Clark, M.S.,

Naval Surface Warfare Center, Dahlgren Division; Department of Homeland Security

Introduction

In 2003, the Department of Homeland Security (DHS) deployed a nationwide bio surveillance system to provide early warning of a biological attack by conducting surveillance for aerosolized biological agents in specific locations across the United States. This effort operates in direct support of DHS Strategic Goals and Homeland Security Presidential Directive 10. DHS requested Naval Surface Warfare Center Dahlgren Division (NSWCDD) demonstrate the established system's ability to collect, detect and identify the presence of a bio-aerosol.

Objectives

Objective #1

Demonstrate that the currently fielded bio surveillance system can detect an intentional aerosol release of a biological simulant in an operationally relevant environment

Objective #2

Demonstrate current modeling capabilities in an operationally relevant environment including up-front collector siting and back-end event reconstruction.

Operationally Relevant

- Draws a distinction between the Naval Support Facility Dahlgren (NSF-Dahlgren) venue and other traditional biological detection test venues or laboratory test facilities
- NSF-Dahlgren offers more relevant operational factors:
  - Environment - Littoral
  - Topography
  - Infrastructure

- NSF-Dahlgren was considered to be more representative of an urban setting and moderate climatic conditions as compared to the typical alternative test venues.

Environmental Impact Statement

The Final Environmental Impact Statement (EIS) Outdoor Research, Development, and Test and Evaluation Activities, Volume 1, prepared by the US Department of the Navy, NSWCDD evaluated the effects of expanding research, development, test and evaluation (RDT&E) activities within the Potomac River Test Range and Explosives Experimental Area complexes, the Mission Area, and special-use airspace at NSF-Dahlgren. One of those activities specifically covered is the release of biosafety level 1 biological simulants. This demonstration is covered by the National Environmental Policy Act by the 2013 RDT&E EIS, Record of Decision signed 10/15/1013.

Simulant

Bacillus atrophaeus var. globigii (aka Bg)
Dugway Bg ATCC #9372 amended with 5% Aerosil R 812S Fluidizer

- Provided by Dugway Proving Ground (DPG)
- Biosafety Level 1 (BSL-1) organism
- Ubiquitous in the environment, commonly found in hay, soil and water.
- Cultured Bg creates pigmented colonies that allow for discrimination from other environmental bacteria.
- Light Microscopy
  - Large number of single phase bright Bg spores with many large >20µm conglomerations

- Scanning Electron Microscopy
  - Estimated spore size 0.6-0.8µm x 1.1-1.4 µm
- Laser Diffraction Analysis
  - Number % vs. Particle Size
  - Data aggregated from 0.6-1.4 µm region
    - 33% of material fell within this region
  - Within that region the mean particle size was 0.76 µm with a median of 0.71 µm.
- Serial Dilution Plating
  - 3.94E+07 CFU/mg

Reference System Characterization

Referee System and Dissemination System Development and Characterization

- Completed inside negative pressure isolation tent with adjustable unidirectional airflow.
- Objectives of initial indoor characterization:

#1. Demonstrate the ability to create and control biological aerosol
  - ➢ Effectively aerosolize a powder to achieve predetermined concentrations

#2. Collect/detect the aerosolized Bg using the DHS system
  - ➢ Assess system performance with selected simulant – qualitative

#3. Collect/quantify the aerosolized Bg using the referee system
  - ➢ The ruler by which the system under test's performance is measured - quantitative

Collector Siting

- Site selection performed as it is operationally for a selected jurisdiction. NSF-Dahlgren installation was treated as a representative jurisdiction and ten collection sites were identified around the installation.
- As a follow on to the model's output for collector siting, an operational process called micro-siting was performed by the program office where collector location was adjusted to ensure they were in non-hazardous locations reasonably close to a power source if possible.

Collection Site Set-Up

Equipment

Filter Sampling Equipment

Viable Particle Collector

Weather Data Collection

Particle Sizers and Counters

Filter Sampling Equipment served as the ground truth data for each trial. The AGI and two different particle counters offered additional supporting data and the weather data equipment was very useful for deciding when to release and for root cause investigations when simulant was detected in unexpected locations due to re-aerosolization.

Dissemination

- Powder dispersal conducted using the Fox Venturi Eductor and compressed nitrogen.
- Dissemination stand built to be mobile for maximum flexibility
- Trial matrix ranged from 100–300 grams
- Point and moving point releases
- Co-located partial mobile referee system
- Fluorescent tracer powder – Tinopal® OB
  - Began using after the first few trials to leverage the IBAC as a tool to better track simulant cloud arrival times and duration.

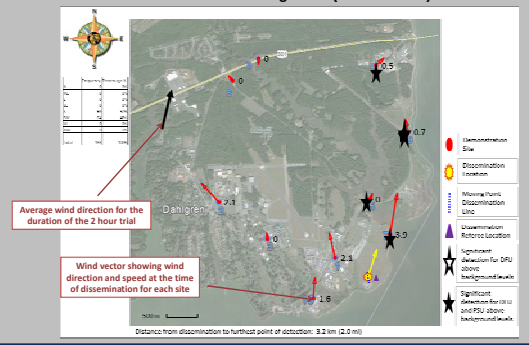
Trial #	Amount of BG	Dissemination Type and Distance Traveled (if Moving Point)
Pilot 1	100 grams (g)	Point Source
Pilot 2	250 g	Moving Point Source; 300 meters (m)
1	150 g	Point Source
2	150 g	Moving Point Source; 150 m
3	150 g	Moving Point Source; 300 m
4	150 g	Point Source
5	150 g	Moving Point Source; 150 m
6	150 g	Moving Point Source; 300 m
7	300 g	Moving Point Source; 300 m
8	250 g	Moving Point Source; 150 m
9	300 g	Moving Point Source; 300 m
10	100 g	Moving Point Source; 300 m
11	250 g	Point Source
12	300 g	Point Source
13	300 g	Point Source
14	100 g	Moving Point Source; 150 m
15	150 g	Moving Point Source; 150 m
16	250 g	Moving Point Source; 150 m
17	200 g	Moving Point Source; 150 m
18	200 g	Moving Point Source; 150 m
19	150 g	Moving Point Source; 150 m
20	125 g	Moving Point Source; 150 m
21	100 g	Moving Point Source; 300 m
22	250 g	Moving Point Source; 150 m
23	150 g	Point Source
24	100 g	Point Source

Design and Execution

- Outdoor Background Characterization
  - Assessment of levels of native Bg present in the environment prior to beginning Bg releases
  - Total of three weeks in duration – 2 months prior, 1 week prior and 1 week following pilot testing
  - 12 and 24 hour sampling periods
  - Native Bg detected at very low levels at 6 of 10 sites at least once during three week background period.
- Trial Execution
  - Two hours in duration
    - Time determined by preliminary modeling that showed the cloud taking 45-60 minutes to traverse the installation with low wind speeds as worst case scenario.
    - This time was doubled to ensure that the entire simulant cloud could be captured.
  - Trial start was proceeded by a mandatory one hour background collection to be used as a baseline for comparing trial samples to following the release of simulant
  - The two hour trial started at the time of dissemination of the simulant.
- Laboratory Processing of Samples
  - Samples (background and trial) were processed and analyzed in parallel immediately following delivery
  - Field spikes and blank filters were run in parallel for QA/QC purposes.

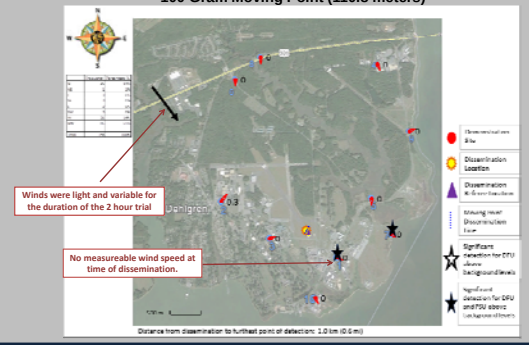
Trial 20140904

300 Gram Moving Point (107.6 meters)



Trial 20140930

100 Gram Moving Point (110.8 meters)



Summary

Trial execution was completed from 19-August until 8-October 2014.

- A total of 24 trials were executed with 21 being accepted trials
- Successful detection in 20 of 21 accepted trials
  - Of which over 50% had positive detections at more than one site.
- Positive detections were recorded up to 2.1 miles away from the point of dissemination.

The Field Demonstration was executed successfully. The main objective of demonstrating the currently fielded bio-surveillance system could detect an intentional biological aerosol release was met.

Data was collected and provided to LANL for the second objective of demonstrating the existing modeling capabilities for site selection and event reconstruction.

Trial #	Date Executed	Amount of BG	Dissemination Type and Distance Traveled (if Moving Point)	Trial Accepted Yes/No
Pilot 1	7/28/2014	100 grams (g)	Point Source	Yes
Pilot 2	7/31/2014	250 g	Moving Point Source	Yes
1	8/19/2014	150 g	Point Source	Yes[1]
2	8/20/2014	150 g	Moving Point Source; 141.8 meters	Yes[1]
3	8/21/2014	150 g	Moving Point Source; 121.5 meters	Yes
4	8/24/2014	150 g	Point Source	No
5	8/25/2014	150 g	Moving Point Source; 137.2 meters	No
6	8/27/2014	150 g	Moving Point Source; 140.6 meters	Yes
7	8/28/2014	300 g	Moving Point Source; 186.5 meters	Yes
8	9/3/2014	250 g	Moving Point Source; 129.0 meters	Yes
9	9/4/2014	300 g	Moving Point Source; 107.6 meters	Yes
10	9/9/2014	100 g	Moving Point Source; 87.7 meters	Yes
11	9/10/2014	250 g	Point Source	Yes
12	9/11/2014	300 g	Point Source	Yes[4]
13	9/15/2014	100 g	Point Source	No[3]
14	9/16/2014	100 g[2]	Moving Point Source; 111.3 meters	Yes[4]
15	9/18/2014	150 g	Moving Point Source; 162.8 meters	Yes[1]
16	9/21/2014	250 g[2]	Moving Point Source; 219.8 meters	Yes[4]
17	9/22/2014	187.5 g[2]	Moving Point Source; 164.8 meters	Yes[1]
18	9/23/2014	200 g[2]	Moving Point Source; 215.0 meters	Yes[4]
19	9/25/2014	150 g[2]	Moving Point Source; 196.4 meters	Yes[4]
20	9/29/2014	125 g[2]	Moving Point Source; 117.9 meters	Yes[4]
21	9/30/2014	100 g[2]	Moving Point Source; 110.8 meters	Yes[4]
22	10/2/2014	250 g[2]	Moving Point Source; 133.2 meters	Yes[4]
23	10/8/2014	150 g[2]	Point Source	Yes
24	10/8/2014	100 g[2]	Point Source	Yes

[1] Tinopal® (OB powder) was also disseminated.
[2] Site 8 DFU and PSU samples were omitted from trial due to possible contamination.
[3] Site 1 DFU and PSU samples were omitted from trial due to possible contamination.
[4] Site 5 DFU and PSU samples were omitted from trial due to possible background issues.

Acknowledgements

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Life Sciences Dual Use Research of Concern (DURC)

Brendan Doyle[1], Cayce Parrish[1], Eli Walton[2]

[1]U.S. Environmental Protection Agency; [2] Contractor, U.S. Environmental Protection Agency

www.epa.gov

Introduction

Life sciences research is essential to scientific advances that underpin improvements in public health and safety, agriculture (including crops and other plants and animals), the environment, materiel, and national security.

Despite its values and benefits, certain types of research conducted for legitimate purposes can be utilized for both benevolent and harmful purposes. Such research is called *dual use research*.

Dual Use Research of Concern (DURC):

A subset of dual use research defined as life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.

Background

In 2011, two studies of avian flu virus, funded by the National Institutes of Health, raised national security concerns when researchers tried to publish their results, which included methods for making the virus more transmissible. This research had the potential to meet the definition of *dual use research of concern*.

In response, the U.S. Government issued two government-wide policies for the oversight of life sciences research involving avian flu virus and 14 other high-consequence agents and toxins:

- Dual Use Research of Concern (DURC) Policy; and, the
- Institutional Dual Use Research of Concern (iDURC) Policy.

DURC and Institutional DURC Policies

Two policies have been released that seek to enhance oversight, ensure responsible research, and mitigate the risks associated with *dual use research of concern*

United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern (DURC Policy) March, 2012

- Complements existing regulations and policies governing the possession and handling of pathogens and toxins;
- Sets standards and procedures for departments and agencies to review federally funded or conducted life sciences research involving:
  - 15 agents or toxins;
  - One or more listed experiments; and that
  - Meets the definition of dual use research of concern.
- Requires the implementation of risk mitigation measures, if necessary.

United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (iDURC Policy)
79 Federal Register 57589, effective September 25, 2015.

Sets standards and procedures for institutions that receive funding from the U.S. Government for life sciences research to review all life sciences research conducted at their institution and determine whether it :

- Involves any of the 15 agents or toxins;
- Involves one or more listed experiments;
- Meets the definition of dual use research of concern.

Requires the implementation of risk mitigation measures, if necessary.

EPA Order 1000.19, Policy and Procedures for Managing Dual Use Research of Concern *(approved September 14, 2016)* establishes a systematic approach for Environmental Protection Agency (EPA) and extramural researchers to identify and mitigate when and how the risks that knowledge, information, products, or technologies produced by certain life sciences research may be misapplied in ways that pose significant threats with broad, potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. *EPA's Policy is that all life sciences research involving one or more of the listed select agents or toxins shall be subject to institutional review and oversight and conducted and communicated responsibly.*

"Life Sciences Research," according to EPA, and based on the definition of research in 40 CFR § 26.102(d), is a systematic investigation designed to develop or contribute to generalizable knowledge involving living organisms (e.g., microbes, human beings, animals, and plants) and their products. EPA does not consider the following activities to be research: routine product testing, quality control, mapping, collection of general-purpose statistics, routine monitoring and evaluation of an operational program, observational studies, and the training of scientific and technical personnel. [Note: This is consistent with Office of Management and Budget Circular A-11.]

EPA Responsibilities

DURC Policy

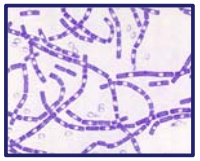
Under the DURC Policy, EPA must conduct a semi-annual inventory of all funded or conducted research that involves any of the 15 agents or toxins.

- The Agency has regularly conducted this survey since June 2012 and reports the results to the National Security Council

iDURC Policy

Under the iDURC Policy, EPA must require institutions that receive life sciences research funding from EPA to comply with the Policy's provisions.

- The Agency has developed a solicitation provision and contract clause for contracts, terms and conditions for grants, and language for interagency agreements and Federal Technology Transfer Act (FTTA) agreements that address institutional compliance with the iDURC Policy.
- **All EPA funding agreements that involve life sciences research must contain language addressing iDURC Policy compliance.**



DURC Agents, Toxins & Experiments

The following 15 agents and toxins are identified in the DURC and iDURC Policies:

1. Avian influenza virus (highly pathogenic)
2. *Bacillus anthracis*
3. Botulinum neurotoxin (in any quantity)
4. *Burkholderia mallei*
5. *Burkholderia pseudomallei*
6. Ebola virus
7. Foot-and-mouth disease virus
8. *Francisella tularensis*
9. Marburg virus
10. Reconstructed 1918 Influenza virus
11. Rinderpest virus
12. Toxin-producing strains of *Clostridium botulinum*
13. Variola major virus
14. Variola minor virus
15. *Yersinia pestis*

Categories of experiments:

a) Enhances the harmful consequences of the agent or toxin;
b) Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification;
c) Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
d) Increases the stability, transmissibility, or the ability to disseminate the agent or toxin;
e) Alters the host range or tropism of the agent or toxin;
f) Enhances the susceptibility of a host population to the agent or toxin;
g) Generates or reconstitutes an eradicated or extinct agent or toxin listed above.

If a research project involves any of these agents or toxins, Principal Investigators and their institutions should notify EPA's Institutional Contact for Dual Use Research at: **DURC@epa.gov.**

U.S. Environmental Protection Agency
Office of Research and Development

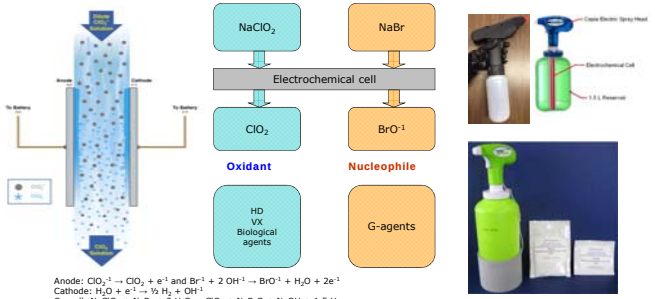
Advanced Decontamination Concepts and National Security Product Development

Brian France (bfrance@tda.com) and William Bell,
TDA Research, Inc.
12345 W 52nd Ave, Wheat Ridge, CO. USA 80033

Handheld Electrochemical Decontamination Technology – $eClO_2$

In this project, TDA Research, Inc. (TDA) is developing a new liquid decon solution based on electrochemical technology originally developed at Procter & Gamble. The system consists of a dispenser with an electrochemical cell in which electrical power applied to an aqueous solution of sodium chlorite produces chlorine dioxide (ClO_2), an effective decontaminant of CW agents that is also highly effective against biological threats, including bacterial spores. A sodium bromide salt in the same solution produces hypobromite ion (BrO^{-1}) during electrochemical conversion, which decomposes the G-agents. Both ClO_2 and BrO^{-1} are highly reactive and not persistent: they react quickly with any CW or BW agents present, and decompose in a short period of time, leaving no hazardous residue. The decontaminant has also been shown to be compatible with military materials.

For electrochemical conversion only solid salts are transported, not reactive species; starting salt solutions can be made with available on-site water. The solid salts are highly shelf stable and readily transportable. Consumables consist of long shelf life lithium batteries and solid salt packages. The electrochemical system is safe for the operator because the active component is generated only as needed, so operators do not mix or carry any highly reactive toxic material. The chlorine dioxide and hypobromite react and decompose within a few hours, leaving no hazardous residue. This system benefits the warfighter by providing an effective decon solution that is readily transported, easy to use, environmentally friendly and safe for the operator.



Anode: $ClO_2^{-1} \rightarrow ClO_2 + e^{-1}$ and $Br^{-1} + 2\ OH^{-1} \rightarrow BrO^{-1} + H_2O + 2e^{-1}$
Cathode: $H_2O + e^{-1} \rightarrow \frac{1}{2}\ H_2 + OH^{-1}$
Overall: $NaClO_2 + NaBr + 2\ H_2O \rightarrow ClO_2 + NaBrO + NaOH + 1.5\ H_2$

Environmental Protection Agency Registration Efficacy Testing

- Efficacy against anthrax spores, by modified AOAC 2008.05 test protocol
- Testing was performed at Naval Surface Warfare Center - Dahlgren Division (NSWCDD)
  - Buhr, T., Young, A., Minter, Z., Wells, C. and Shegogue, D. (2011), Decontamination of a hard surface contaminated with *Bacillus anthracis*ΔSterne and *B. anthracis* Ames spores using electrochemically generated liquid-phase chlorine dioxide ($eClO_2$). Journal of Applied Microbiology. **111**, 1057-1064

Test Organism	Identification Number	Date Preformed	Lot # (Part A – Salts) (Part B – Surfactants)	Results (Log Reduction)		
				1 min.	5 min.	15 min.
Bacillus anthracis ΔSterne	BAC1056	01/22/2010	# 9 and 4	7.0	7.0	7.0
		01/25/2010				
		01/28/2010				
		01/23/2010	# 6 and 6	7.1	7.1	7.1
		01/26/2010				
		01/29/2010				
		01/24/2010	# 11* and 5*	7.1	7.1	7.1
		01/27/2010				
		01/30/2010				

- NSWCDD performed tests similar to the EPA protocols using *Bacillus anthracis* Ames and measure similar performance, 7-log kill within 1 minute.

EPA Registration for Efficacy against Anthrax

- July 23, 2015
- **US EPA Reg. No. 85797-1**

Advanced Surfactant Blend Designed Specifically for Decontamination with Multiple uses – SSDX-12

Aircraft are extremely expensive and sensitive assets which are critical for defense and transportation. Chemical products used on an aircraft must meet strict materials compatibility requirements to ensure they don't degrade, shorten the life expectancy or cause the failure of any aircraft component.

Aircraft that have been contaminated with toxic chemicals cannot be decontaminated using traditional decon solutions, which include oxidants such as bleach or hydrogen peroxide. Using these reactive materials would corrode parts; damaged components would then have to be identified and replaced to prevent catastrophic failure. Decontaminants that remove the toxic chemicals without harming the aircraft are essential.

The goal of this effort was to develop a detergent formulation that was specifically tailored to remove chemical and biological agents from contaminated surfaces, and that had excellent compatibility with all the components of an aircraft. To ensure the product would be successful and widely accepted it was designed as a dual use item, both for decontamination and as an aerospace equipment cleaning compound qualified under military and commercial standards.

- A surfactant blend specifically designed to emulsify and lift agents from surfaces
- Non-reactive, non-corrosive, pH neutral, no-VOC, biodegradable
- High concentration/ HE formulation (reduced shipping and storage)
- Dual use
- Commercially available
- Non-hazardous, no DOT restrictions

Decontamination Performance – HaMMER ATD

Large panel decontamination testing using SSDX-12 as a decontaminant prewash showed

- Low or high pressure SSDX-12 prewash provides improved decontamination compared to no prewash
  - Low pressure prewash was used in the contact hazard testing but could have additional safety benefits to the decontamination operator by preventing backsplash.
- Scrubbing during decontamination is not required if SSDX-12 prewash is performed
- SSDX-12 prewash removes 99.95% of a VX contact hazard without reactive decontaminants
- SSDX-12 used prior to a reactive decon during VX decon allows for decontamination to below 0.75 mg/m2 requirements (target objective <0.75 mg/m²)
- SSDX-12 prewash removed 6 times more VX than a water prewash
- A 30 minute SSDX-12 prewash can bring the HD contact hazard down from 10g/m2 to below the 15 mg/m2 requirement without the need for expensive reactive decontaminants. (target objective <100 mg/m²)
- SSDX-12 prewash prevents migration of HD to non-contaminated substrates better than water alone.

Automated Vehicle Decontamination

Compatible with mobile and stationary vehicle decontamination platforms

Aircraft distributor

Unique Challenges of Decontaminating Sensitive Equipment - Routine Aircraft Cleaner

Qualified to the following Aircraft cleaning specifications

- U.S. Air Force MIL-PRF-87937D as a Type IV heavy duty water dilutable aerospace cleaner
  - Currently used to clean C-130's, C-17 and B-52's
- AMS 1626C Cleaner for Aircraft Exterior Surfaces
- AIRBUS AIM09-00-002 External and General Cleaners
- Boeing D6-17487 Exterior General Cleaners
- Douglas Aircraft CSD No.1 Type 1 General Cleaning of Painted and Unpainted Surfaces

Heavy Maintenance Facilities / Industrial Cleaner

Hill Air Force Bases uses the product as a facilities cleaner

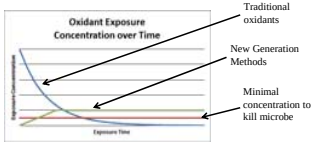
- Industrial wastewater treatment compatible
- Prevents migration of heavy metals in aircraft maintenance facilities
  - OSHA Expanded Standards, Cd and Cr

Photo-Generated Chlorine Dioxide for Consumer and National Security Applications

DHS requires the ability to respond to an attack with biological agents. Specifically, it requires a decontaminant to kill anthrax spores on building interiors, exteriors, and even large outdoor surfaces. TDA is developing an innovative bio-agent decon technology that is effective for decon of building interiors, exteriors, and is particularly suited for decon of wide areas, such as airports. This technology uses a stable salt solution containing sodium chlorite with a photocatalyst that absorbs light; the light-activated process produces chlorine dioxide (ClO2), a known biocide and effective anthrax decontaminant. Chlorine dioxide is reduced back to chlorite after oxidizing an anthrax spore and the photocatalyst can repeatedly absorb light, allowing continued production of ClO2. This makes the technology ideally suited to sustain a low level of chlorine dioxide in solution over a long time, which has been shown to be effective against bacterial spores, biofilms and stains. During this project, TDA is working develop an EPA registered formulation for a product with claims of efficacy against anthrax.

Fundamentals

- Light activated chemistry
- Oxidant/Chlorine Dioxide is not generated until it is applied to the surface
- Lower concentration of chemical components
- Does not require a special applicator
- Not as effective against all chemical agents
- Reduced concentration of chlorine dioxide, begins generating oxidant when applied
- Requires light



Decontamination Performance

- Multiple formulations have shown good efficacy under solar simulation light.
  - 8 log reductions within 15 minutes of solutions of commercially available *B. subtilis* spores
  - Decontaminant is quenched to stop reactions at correct time period
- Surface decontamination is slower
  - Oxidant presumable has to 'dig' through piles of spores
  - Decontaminant is light dependent, lower light levels require longer exposure times

EPA Registration and Efficacy

- EPA has published product performance test guidelines – 810.2100 Sterilants – Efficacy Data Recommendations
  - AOAC Method 966.04 – Sporicidal Activity of Disinfectants Test
  - 15 minute exposure and 7-log exposure challenge
- Formulation optimization requirements to speed up decontamination
- Goal is a formulation that is an increment size of a consumer product (4 consumer packs = 1 national security pack)
  - Preliminary Results
    - 7.34 log reductions, dried on glass slides, 15 minutes exposure
    - 7.34 log reductions, dried on Arizona Test Dust, 15 minute exposure

Potential Consumer Applications

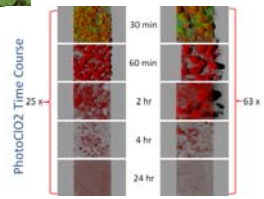
- Non-food contact, hard surface sanitizer
  - Passes ASTM E 1153-03 testing
  - 3 log reduction with 5 minute contact period
    - *Klebsiella pneumoniae* – 99.9992% reduction
    - *Staphylococcus aureus* – 99.9802% reduction
  - Could be EPA approved

- Outdoor Mold and Mildew Remediation
  - EPA registration has been submitted, expected January 2017
- Plant and Crop Protection
  - Plant are susceptible to microbial pathogens too. Very few anti-bacterial products are available
  - Photo-ClO2 is effective against bacterial leaf spot
  - EPA submission has begun, expected November 2017

- Biofilm Control Products

Summary

- EPA registration of a biological decon national security product is
  - Required
  - Not simple
  - Can be successfully accomplished
- Dual use products are more likely to be successful and sustainable
- eClO2 has been successfully registered
- Development and registration of other, dual-use products are in progress



Acknowledgements

- eClO2 funding, Army Research Office, STTR Phase II contract: W911NF-13-C-0096
- Decontamination Surfactant, US Air Force, SBIR Phase II contract: FA8222-14-C-0001
- Photo-catalytic chlorine dioxide, Department of Homeland Security, SBIR Phase II contract: HSHQDC-14-R-00035
- Plant and crop protection, US Department of Agriculture, SBIR Phase I contract:2016-33610-25483

Characterization of Anthrax Surrogates by Chromogenic Media

Douglas W. Hamilton[1] and Paul Lemieux[2]
[1]ORISE Research Participant • [2]United States Environmental Protection Agency • [2]National Homeland Security Research Center

Abstract

The safety threat posed by spores of virulent *Bacillus anthracis* precludes its use in many research activities and planning exercises. Avirulent surrogates belonging to the *B. cereus* group have been reported with properties similar to *B. anthracis*, with some organisms being useful surrogates for evaluation of decontamination processes, and other organisms being useful surrogates for reaerosolization studies. This study compared the growth characteristics of three members of the *B. cereus* group [*B. atrophaeus* subsp. globigii (BG), *B. anthracis* Sterne (BAS) and *B. thuringiensis* var. kurstaki (BTK)] when cultured on media containing chromogenic substrates. Culture-based analytical methods are the "gold standard" for determining the effectiveness of decontamination efforts and to inform response and recovery initiatives. Plate counting on chromogenic agar provides quantitative information about a sample, can determine if a pathogen is viable, and has the potential to improve laboratory surge response by evaluating samples based on biochemical characteristics. Chromogenic agars contain substrates that are enzymatically cleaved during cellular processes, resulting in the accumulation of the cleaved products within the vegetative cell and the ability to visually identify bacterial colonies (by morphology and color) based on substrate utilization. The chromogenic media investigated in this study produce visually striking blue colonies when organisms possessing specific biochemical processes are cultured.

Three media were selected for this study, including tryptic soy agar (TSA), R&F anthracis chromogenic agar (ACA) and Brilliance *Bacillus cereus* agar (BBCA). In replicate experiments, it was determined that no statistical difference exists between sample enumeration on TSA, ACA and BBCA for the selected surrogate spores. Incubation temperature and time were found to be critical to the positive identification of surrogate spores on chromogenic agars. This study identified methods useful in the characterization and quantification of surrogates routinely used for research and planning activities. The chromogenic agars are useful tools in the differentiation of certain types of spores based on biochemical characteristics, particularly in the presence of background flora that do not grow blue colonies. These media are readily adaptable to standard laboratory analytical methods.

Introduction

Background:

Three media were selected for this study, including tryptic soy agar (TSA), R&F anthracis chromogenic agar (ACA) and Brilliance Bacillus cereus agar (BBCA). TSA is a general nutrient agar and served as the control media for statistical analyses. ACA is a selective and differential media that contains the chromogenic substrate 5-bromo-4-chloro-3-indoxyl-choline phosphate (X-CP) which identifies the production of phosphatidylcholine-specific phospholipase C (PC-PLC) produced by BAS and BTK resulting in blue colony formation. A mutation in the plcR gene of BAS inactivates the regulator, PlcR, reducing PC-PLC activity and allowing BAS to be differentiated from BTK following an additional 24 hours of incubation. BBCA is a differential media that contains the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-glucopyranoside which identifies the production of β-glucosidase resulting in blue colony formation.

Objectives:

- Evaluate the enzymatic expression (blue colony phenotype) of three common anthrax surrogates using three types of agar growth media.
- Determine if there is a difference in the number of colonies formed based on the type of media used to culture the spores.
- Recommend useful strategies for the culture-based characterization of each spore type.

Materials and Methods

Organisms

B. atrophaeus subsp. globigii (BG)
B. anthracis Sterne 34F2 (BAS)
B. thuringiensis var. kurstaki (BTK)

Media

Tryptic Soy Agar (TSA)
Brilliance Bacillus cereus Agar (BBCA)
R&F anthracis Chromogenic Agar (ACA)

Methods

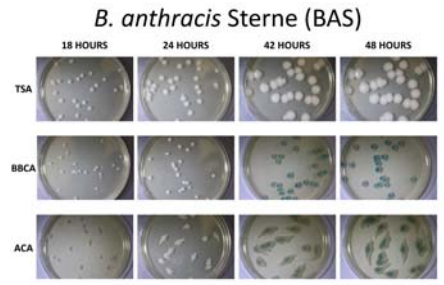
Serially dilute spore stocks
Spread plate 100 µl on media
Incubate @ 36 °C for 48 hours

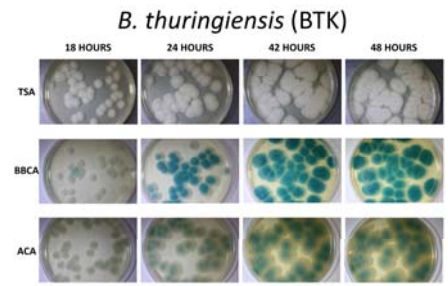
Analysis

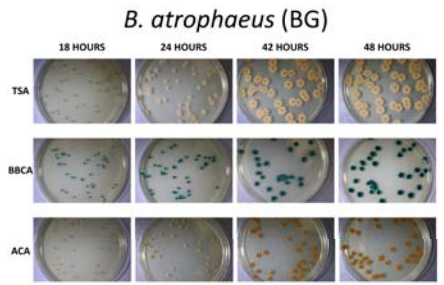
Compare colony formation on chromogenic media to TSA
(t-test with α = 0.05)

Results

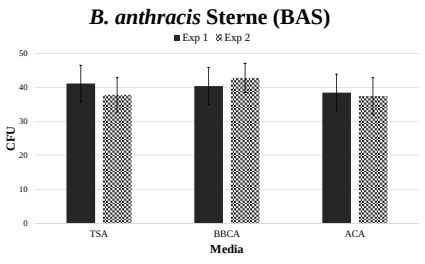
Colony Formation Over Time



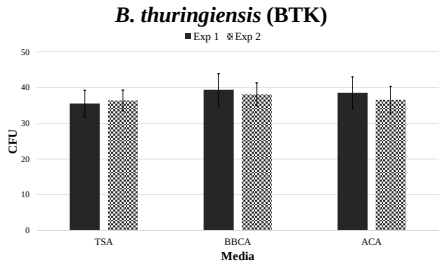




Enumeration of Spores



Replicate enumeration experiments showing no difference between colony formation on control (TSA) and chromogenic (BBCA & ACA) agars [t-tests (α = 0.05)]. (n=5)



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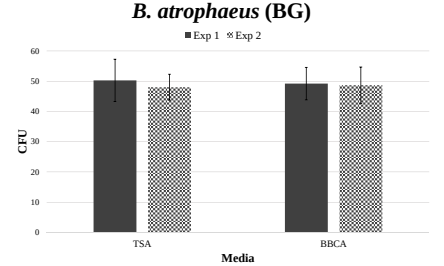


Replicate enumeration experiments showing no difference between colony formation on control (TSA) and chromogenic (BBCA) agars [t-tests (α = 0.05)]. (n=5)

Discussion

Recommended spore enumeration strategies
- BAS: BBCA or ACA at 48 hours & 36 °C
- BTK: BBCA or ACA at 24 hours & 36 °C
- BG: BBCA or TSA at 24 hours & 36 °C

- Enzymatic expression (blue colony phenotype) is temperature dependent.
- Lower incubation temperatures yield negative results for BAS & BTK on both chromogenic media.
- Colony development of BAS is slower on ACA (contains antibiotic).

Acknowledgments

D.W.H. was supported through an appointment to the Research Participation Program at the U.S. Environmental Protection Agency, National Homeland Security Research Center, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the Environmental Protection Agency.

This poster has been subjected to the agency's peer and administrative review and has been approved for publication. The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use.

Broad-Spectrum Enzyme-Based Decontamination of Chemical Warfare Agents: A Flexible Decontamination Platform

Anna M. Leech, Jessica L. Milke, Jonita G. Gidel, Scott Donahue, and Jeremy P. Walker, Ph.D.

FLIR Detection, Inc. Pittsburgh, PA

Limitations of Current State of the Art

- Many commercial off the shelf (COTS) decontaminants have the following deficiencies which limit broad use:
  - Poor materials compatibility (corrosive, caustic)
  - Large footprint for transport / storage
  - Potential inability to transport via air
  - Non-specific decon can produce toxic byproducts
  - Toxic off-gassing
  - Inadequate surfactant packages to extract agents from coatings, plastics, elastomers

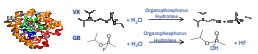
- Past enzymatic decontaminant limitations:
  - Poor enzyme kinetic chemistry for V-series Agents or HD
  - Poor shelf-life work investment
  - Lack good solvent package that is compatible with enzymes to solubilize and extract HD, V-series from challenging surfaces

Advantages of an Enzyme-Based Decontaminant

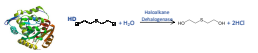
- Recent improvements in catalytic efficiency
  - Genetically engineered organophosphorous hydrolase (OPH) enzymes will hydrolyze both G and V-series agents
    - V-series agents are cleaved at the P-S bond, eliminating byproduct toxicity (EA2192)
- No need for agent-specific formulations
  - Combining G and V-series hydrolase with haloalkane dehalogenase for sulfur mustard decontamination produces a broad-spectrum chemical agent decontaminant
- Removal of persistent agent from challenging surfaces
  - Enzyme-compatible surfactant formulation
  - Reduces toxic off-gassing after decontamination procedure
- Green Technology
  - Improved economies of scale for protein production
  - Benign components
  - Low environmental impact & low occupational health hazard
- Low logistical burden
  - Catalytic formulations require less mass than stoichiometric ones

Enzyme Specificity for Broad-Spectrum Decontaminant

OPH variant enzyme 3D structure (left) and hydrolysis reactions for the detoxification of VX (top) and GB (bottom). The enzyme functions optimally at pH ~ 8. Acidic byproducts generated from hydrolysis are neutralized by buffers.



Haloalkane DHG 3D protein structure (left) and hydrolytic dealkylation of sulfur mustard (right). Non-toxic thiodiglycol and hydrochloric acid byproducts are generated. These byproducts are buffered by salts present in FLIR's decontaminant.

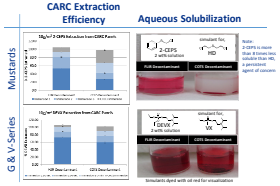


Agent Decon Kinetics for OPH	
Agent	k_{cat}/K_m (M^{-1} min^{-1})
Tabun (GA)	1.58×10^8
Sarin (GB)	1.48×10^8
Soman (GD)	8.25×10^6
Cyclosarin (GF)	4.57×10^6
VX	4.99×10^6 (wild-type OPH) 3 x 10^9

Tsai, et. al. (2012) *Biochemistry* 51: 6463.
Bigley, et. al. (2013) *J. Am. Chem. Soc.* 135: 10426.
Cherny, et. al. (2013) *ACS Chem. Biol.* 8: 2594.

Formulation for Solubilization of Persistent Agents

- The biggest challenge for Decon is removing the agent from porous surfaces:
  - CARC paint
  - SBR rubber, elastomers
  - Acrylics
- FLIR emulsion outperforms commercial off-the-shelf (COTS) decontaminant in solubilization & extraction of persistent simulants
- Additional Features:
  - Enzyme compatible
  - Minimal agitation required
  - Stable for > 24 hours



ASTM Materials Compatibility

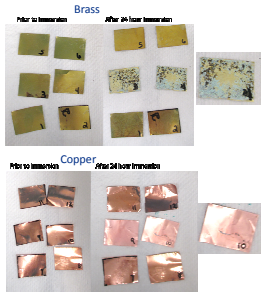
- FLIR decontaminant was tested side-by-side with a commercial off the shelf (COTS) oxidative decontaminant
- FLIR decontaminant underwent materials compatibility testing
  - ASTM methods were followed for metal corrosion (top right), sorption and hardness for various plastics and elastomers (bottom right)
- Compatibility testing demonstrate that FLIR's decontaminant is not damaging to these materials
  - While COTS decontaminate severely corrodes some metals

	FLIR Decontaminant		COTS Decontaminant	
Material	Corrosion Rate (mm/y)	Rating	Corrosion Rate (mm/y)	Rating
Aluminum 1100	0.01	Excellent	1.2	Excellent
Aluminum 2024	0.02	Excellent	0.1	Excellent
Aluminum 6061	0	Excellent	0.5	Excellent
Aluminum 7075	0.01	Excellent	0.8	Excellent
Stainless Steel 302	0	Excellent	0	Excellent
Stainless Steel 304	0	Excellent	0	Excellent
Stainless Steel 316	0	Excellent	0	Excellent
Stainless Steel 321	0	Excellent	0	Excellent
Stainless Steel 410	0	Excellent	0	Excellent
Carbon Steel 1018	0	Excellent	0	Excellent
Blue Stainless Steel 1095	0	Excellent	0	Excellent
Brass	0.04	Excellent	105	Severe
Copper	0.08	Excellent	95	Severe

	Sorption		Hardness	
Material	% Mass Δ (grams)	Rating (failure: 5%)	% Hardness Δ (durometer units)	Rating (failure: 2%)
Polycarbonate	0.137	pass		
Acrylic	0.025	pass		
BUNA N	0.439	pass	1.852	pass
EPDM	0.178	pass	0.926	pass
Silicone	0.211	pass	-7.054	threshold
Viton	0.041	pass	0	pass
SBR	0.124	pass	0.167	pass
Neoprene	-0.279	pass	0	pass

ASTM Materials Compatibility: Corrosion Testing

- COTS decontaminant (coupons 3 & 4) causes significant corrosion to brass, while FLIR decontaminant (coupons 5 & 6) has excellent material compatibility
- COTS decontaminant causes significant corrosion to copper (coupons 9 & 10).
  - A color change occurs, and a hole develops in the metal after immersion in COTS decontaminant.
- FLIR decontaminant (coupons 11 & 12) has excellent material compatibility with copper

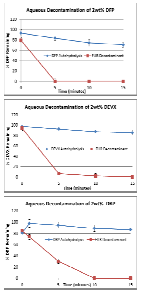


Aqueous Decontamination: Stirred Reactor Decon

- Addition of enzymatic action to the FLIR emulsion formulation results in rapid simulant decontamination in <15 minutes

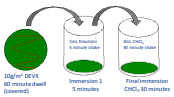
% Decontamination			
Time (min)	DFP Decon	DEVX Decon	DBP Decon
0	25%	0%	10%
5	99.9%	94%	69%
10	99.9%	99.1%	99.9%
15	99.9%	99.9%	99.9%

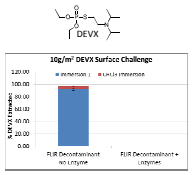
After 15 minutes, >99.9% DFP, DEVX and DBP are detoxified by FLIR decontaminant!



Surface Decontamination: Extraction & Decon

- Liquid Challenge: 10g/m² contact
- Chemical agent resistant coating (CARC) panels were challenged with 10g/m² DEVX and allowed to dwell for 60 minutes
- FLIR's enzymatic decontaminant is capable of solubilizing, extracting, and detoxifying liquid agents residing on or soaked into surfaces.





After 5 minute immersion, >99.999% DEVX is detoxified by FLIR decontaminant!

Enzymatic Performance Specifications

- Liquid challenge: 10g/m² contact

Joint General Purpose Decontaminant Requirements

Agent	Simulant Tested	Threshold Level		Objective Performance		Current Levels	
		Conc.	Time	Conc.	Time	Conc.	Time
Nerve-G	DFP	16.7mg/m²	30 min	10mg/m²	5 min	0.1mg/m²	5 min
Nerve-V	DEVX	0.4mg/m²	30 min	0.3mg/m²	5 min	0.1mg/m²	5 min
Blister-H	n/a	33mg/m²	30 min	15mg/m²	5 min	10mg/m² (with Blister-H)	15 min

FLIR decontaminant exceeds objective performance specifications with G- and V-series nerve simulants!

Flexible Decon Formulations

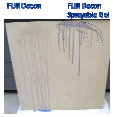
Personal Decon
- The components have been utilized in common household or beauty items
- Replace charcoal-based sorptive decontaminants & RSDL

Aircraft Decon
- Sufficient material compatibility to detoxify aircraft and sensitive equipment

Immediate Decon
- Fast-acting sprayable formulation due to the rapid hydrolysis of simulants

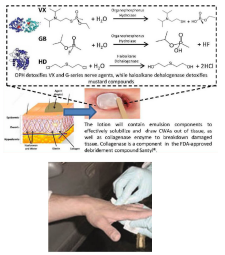
Human Remains Decon
- Non-corrosive formulation can be applied and remain active for extended time courses

Clearance Decon
- Sprayable gel formulation which decontaminates surfaces and off-gassed vapors over 72 hrs. through the addition of polymers to enhance physical properties



Skin Decontaminant Features

- Benign components
  - Nonenhancement of percutaneous agent absorption
  - Nontoxic and noncorrosive components
- Components easily integrated to gel or cream
  - Ease of application by hand to affected areas
- Neutralization of CWAs
  - Rapid hydrolysis of G- and V-series, and HD sulfur mustard
  - Hydrolysis products are non-toxic
- Enhanced Stability
  - Shelf-life > 3 years



Path Forward

- Complete productization of decontaminant
- 3rd-Party live agent validation of performance
- Create TDP and product marketing literature
- Evaluate surfactant extraction performance with skin surrogates
- Test for skin allergenicity

Acknowledgements

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- Prof. Frank Raushel
  - Texas A&M University

Contact:
Anna M. Leech
P: +1 412 423 2100 x 101
Email: anna.leech@flir.com | www.flir.com

FLIR Detection
2240 William Pitt Way, Pittsburgh, PA 15238, USA

Pre-Treatment Technologies to Facilitate Management of Animal Carcasses from Animal Health Emergencies

Sandip Chattopadhyay, Paul Lemieux; US EPA, Office of Research and Development
Lori Miller; USDA, Animal and Plant Health Inspection Service

United States Environmental Protection Agency

Large-Scale Mobile Carcass Grinder

Carcass in Digester

Alkaline Hydrolysis Unit

Table 1. Carcass Pretreatment Options Matrix

Disposal Option	On-site Size Reduction	Digestion	Bioreduction	Alkaline Hydrolysis	Sterilization	Freezing	Physical Inactivation	Chemical Inactivation	Additives/ Sorbents	Encapsulation	Packaging
Rendering	+++	++	++	-	++	++	++	++	++	-	-
Incineration	+++	+	+	-	+++	++	++	++	+++	+	++
Composting	+++	+++	+++	-	-	++	++	-	+++	-	-
Burial	++	+	-	+	+++	++	++	++	+++	++	++
Burning	+++	-	-	-	+++	-	++	++	+++	+	++
Landfill	++	+	-	+	+++	++	++	++	+++	++	++

Color Key: Ideal; Subject to acceptability of characteristics of feedstock by the processing facility/plant; Not Suitable

Note: Several of the pretreatments may have overlapping processes. Some of the activities can be conducted at centralized or mobile locations. +++, ++ and + denote qualitative importance of the criteria (+++ > ++ > +), and – indicate not applicable.

Table 2. Comparison of Pretreatment Technologies and Overall Ranking against Various Carcass Management Options

Criterion	No Pretreatment	Size Reduction (1)	Size Reduction + Physical Inactivation	Size Reduction + Chemical Inactivation		
Chemical Inactivation Agent				Acid (Citric)	Bleach (Hypochlorite)	Oxidizing Agent (Virkon-S)
Overall protection of human health and the environment	2	3	4	4	1	3
Compliance with ARARs (2)	5	3	5	5	3	4
Long-Term Effectiveness and Permanence	3	3	5	5	5	5
Reduction of Toxicity, Mobility, and volume through Treatment	3	4	5	5	5	5
Short Term Effectiveness	2	3	4	4	4	3
Implementability						
Rendering (3)	4	5	5	3	2	3
Burial	5	3	3	3	2	3
Landfill	3	4	5	4	3	4
Composting	4	5	4	4	3	4
Incineration	2	5	3	4	2	3
Burning	2	5	3	4	2	3
Cost	5	4	2	3	2	1
State Acceptance (4)						
Community Acceptance	3	3	4	4	3	4
TOTAL SCORE						
Rendering	27	28	34	33	25	28
Burial	28	26	32	33	25	28
Landfill	26	27	34	34	26	29
Composting	27	28	33	34	26	29
Incineration	25	28	32	34	25	28
Burning	25	28	32	34	25	28

1 = very negative; 2= negative; 3 = neutral; 4 = positive; 5 = very positive

Digestion
- Liquefy carcasses under acidic conditions
- Uses lactic acid or phosphoric acid
- Releases methane, CO_2, water

Bioreduction
- Biodegradation in partially sealed vessel under mild heat and aeration

Alkaline Hydrolysis
- Hydrolysis in NaOH or KOH under moderately high (150 °C temperatures
- Produces aqueous hydrolysate that requires neutralization
- May or may not be able to be discharged into wastewater systems

On-Site Size Reduction
- Manual of mechanical cutting, grinding, crushing carcass
- Reduce size and/or volume to ease handling and further processing

Sterilization
- Destroying infectious agents using high temperature steam
- Kills all microorganisms

Packaging
- Encase carcass in flexible or rigid container
- Contain liquids
- Limit potential spread of pathogens during transport

Freezing
- Done in fixed facilities or mobile units
- May not impact pathogens, but can be effective in extending storage time and easing transportation

Encapsulation
- Encase carcass in protective matrix
  - Foams
  - Concrete
  - Flyash
  - Lime
- Limit potential spread of pathogens during transport

Additives/sorbents
- Promote oxygen transport
- Dissuade vermin
- Absorb excess liquid

Chemical Inactivation
- Destroying infectious agents using chemical agents (e.g., hypochlorite, peroxide)
- Can be coupled with other pre-treatment approaches (e.g., size reduction)
- Could be limited to surface inactivation

Physical Inactivation
- Destroying infectious agents (other than spores, prions) using high temperature steam
- Effective on limited number of agents (viruses)

Handling and Processing of Carcasses

EPA Technical Fact Sheet - EPA/600/F-16/111
Identification and Screening of Infectious Carcass Pretreatment Alternatives - EPA/600/R-15/053
Feasibility of Selected Infectious Carcass Pretreatment Technologies - EPA/600/R-15/301

Table 3. Advantages and Disadvantages of Carcass Pretreatment Technologies

Pretreatment	Advantages	Disadvantages
On-site Size Reduction	Mobile on-site Low environmental impact Very high throughput capacity Few safety issues for operators Ease in handling and transport of processed material Accelerated decomposition	• Cost of capital equipment • Operating cost of machinery • Potential aerosol production • Groundwater contamination if untreated effluent is released • Soil pollution if carcasses accumulate on the ground faster than the processing rate
Digestion	Long-term storage Kills pathogenic bacteria Cost of storage is relatively low compared to cold storage Potential for increased biosecurity Produces several co-products: biomethane, combined heat and power, compressed natural gas, soil amendments	• Transportation risk if digester is not available on site • TSE agent not inactivated; lactic acid fermentation may fail • Capacity is relatively low • Carcass pre-processing, such as grinding, is recommended • Higher capital cost than composting • Operation requires skilled technicians
Bioreduction	Bacterial load may be significantly reduced/eliminated Minimize need to transport carcasses Provides a method for storing carcasses thereby reducing the number of collections and transports Overall biomass is reduced	• Only research data are available for small-scale operations • Not known to destroy prions (e.g., TSE agents) • Geographical location/terrain limits where can be installed • May require additive (wood chip) to reduce odor/leaching • No commercially available units were identified
Alkaline Hydrolysis	Inactivation of viruses, bacteria, spores, and TSE agents Sterilization and digestion in one unit Reduction of waste volume and weight by as much as 97% No air emission	• Relatively low capacity • Potential issues with disposal of effluent • High pH of effluent must be neutralized prior to disposal
Steam Sterilization	Inactivates most pathogens Low environmental impact Few safety issues for operators Facilitates safe transport Creates value-added product	• High capital cost • Requires pre-configured and constructed systems • An inadequate shredder might retard efficiency • Significant fuel and water logistical requirements • Might not inactivate TSE agents
Freezing	Mobile and on-site freezing facilities are available Increases biosecurity for transportation Prolongs storage for delayed disposal Low cost rental units are available Allows flexibility of choosing one or more disposal options	• Mobile units may not be feasible for large animals • Thawing step required before further processing • Limited bacterial reduction • Energy cost and overall operating cost can be high
Physical Inactivation	Equipment readily available Moderate equipment cost Moderate safety issues Potential for reduction of surface infectious agents Low environmental impact	• Some steam applications alone do not reduce surface bacteria • Significant wastewater/environmental impact • Potential for aerosolizing infectious agents • Slow/labor intensive, and it only removes surface pathogens
Chemical Inactivation	Commercially available Ease of application with little training of personnel Flexible to apply on site or centralized facility in combination with grinding	• Environmental concerns on spillage and final disposal • Surface treatment might not be effective • Some of the chemicals can be harmful • May be significant storage/effluent treatment requirements
Additives/ Sorbents - Natural Organic Sorbents	Sustainable and low environmental impact Enhancement of burial, landfill, composting, incineration Few safety issues for operators Facilitates safe transport Low to moderate cost per carcass	• Does not inactivate infectious agents • Hard materials (wood chips) might not be able to be rendered • Increase in volume of material may increase disposal cost
Additives/ Sorbents - Inorganic Sorbents	Low environmental impact Enhances efficacies of burial and landfill Moderately safe for operators Facilitates safe transport/disposition of carcass material Moderate cost per carcass	• Eliminates rendering as a disposal option • Does not inactivate infectious agents • Unknown impact on composting • May not be appropriate for subsequent combustion disposal
Additives/ Sorbents - Commercial (Chemical) Sorbents	Some active ingredients can kill pathogens Low environmental impact Enhances efficacies of burial, landfill, and incineration Moderately safe for operators	• Some additives may negatively impact rendering/composting • High cost • Some additives do not inactivate infectious agents
Encapsulation	Both mobile and on-site treatment facilities are available Can prevent disease spread during transport Pathogen inactivation possible	• High cost per carcass • Low throughput • No significant pathogen inactivation
Packaging	Mobile and on site packaging are available Low environmental impact Moderate throughput capability Few safety issues for operators Moderate cost per carcass	• May require unwrapping of carcasses prior to disposal • Potential for leakage • Does not reduce infectivity

Table 4. Favorable Applications of the Pretreatment Options

Pretreatment Options	Favorable Applications
On-site Size Reduction	Grind carcasses to reduce size for transport and to use in subsequent processes such as composting, rendering, and digestion; high throughput applications.
Alkaline Hydrolysis	Destroys prions; reduces waste volume and weight by as much as 97%. However, it generates significant amount of liquid waste that require additional treatment.
Steam Sterilization	Sterilizes for shredded mass.
Encapsulation	Safe handling; protection of the immediate environment (not during process of wrapping).
Digestion	Reduce total volume under certain conditions and may take long time.
Additives/Sorbents	Enhance or accelerate disposal processes.
Bioreduction	Reduce total volume and some bacterial pathogens; dispose of animals over time without the need to transport off site; effective for small quantities of biomass.
Freezing	Delay decomposition; safe transportation; large capacity transport to disposal site(s); decontamination of freezer may be necessary.
Inactivation	Eliminates most pathogenic microorganisms for safe transport and handling.
Packaging	Safe transportation to disposal site; safe handling.

Provisional Advisory Levels (PALs): A Tiered System of Exposure Evaluations

John C. Lipscomb, Kevin Garrahan and Tonya Nichols

National Homeland Security Research Center, U.S. Environmental Protection Agency, Cincinnati, Ohio and Washington, DC.

John Lipscomb lipscomb.john@epa.gov

TYPES OF QUESTIONS PALs CAN ANSWER

Why can't Regulatory Standards or Reference Values be used as emergency and temporary exposure guideline values?

What effects can occur from short term exposures?
Can analytical methods detect levels that are harmful?
Are the fastest /cheapest methods sensitive enough to detect potentially harmful concentrations? If not, how much harm might occur from exposures below detection limits?
Can solubility limits prevent the likelihood of some high-risk exposures?

What level of harm might occur at taste or odor threshold concentrations?
If sensory irritation occurs, how much would exposures have to increase before health effects worsen?

WHY ARE PALs NECESSARY?

- Humans are exposed to chemicals via intentional and unintentional actions.
- Regulatory exposure limits to protect population health, but are not established for all toxic chemicals and can be exceeded.

- Risk Values differ among multiple risk systems based on factors including:
  - Health endpoints, populations groups and duration of exposure covered, calculation methods, level of precision, etc.
  - Intended application of value, including
    - Prioritization for further action
    - Screening values
    - Basis for Exposure Regulation
  - Anticipating responses from unplanned and uncontrolled exposures has benefits:
    - Data used by Emergency Responders for worker and population protection
    - Anticipating responses informs selection of medical countermeasures
- Thus, comparisons of numerical risk values from different risk systems should be fully explained.

PAL TIERS represent the assumed continuous exposure concentrations of a chemical in air or drinking water above which the following effects could occur in the general population, including all ages and sensitive subpopulations:

- PAL1: normal compensatory or reversible responses
- PAL2: serious, irreversible or escape-impairing effects
- PAL3: lethality

KEY CONCEPTS

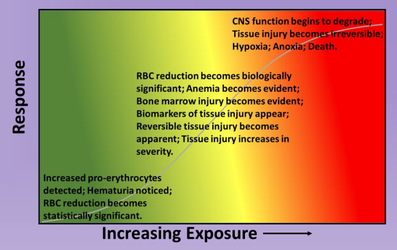
- **P**rovisional: can be updated as newer data become available.
- **A**dvisory: not regulatory, not exclusive in their ability to inform a decision.
- **L**evels: expressed in units of concentration in environmental media.
- Targeted to Risk Assessors, Emergency Responders, On-Scene Coordinators and Emergency Planners.
- Inform decisions related to reoccupation of contaminated infrastructure and reutilization of contaminated resources.
- Focus on Toxic Industrial Chemicals and Chemical Warfare Agents.
- PALs Standing Operating Procedure (Young et al., 2009) derived from SOP for Acute Exposure Guideline Levels (AEGL; NRC, 2001).
  - Tiered risk values: minimal to lethal exposures characterized.
  - Extend coverage to Inhalation exposures beyond 8 hours (to two years).
  - Extends coverage to Oral exposures, with drinking water focus.

PRIORITY CHEMICALS

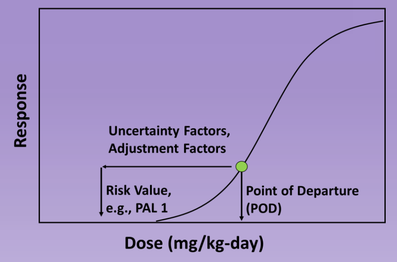
Interim Documents address 130+ Chemicals and 3000+ values, for chemicals identified in several prioritization approaches, including:

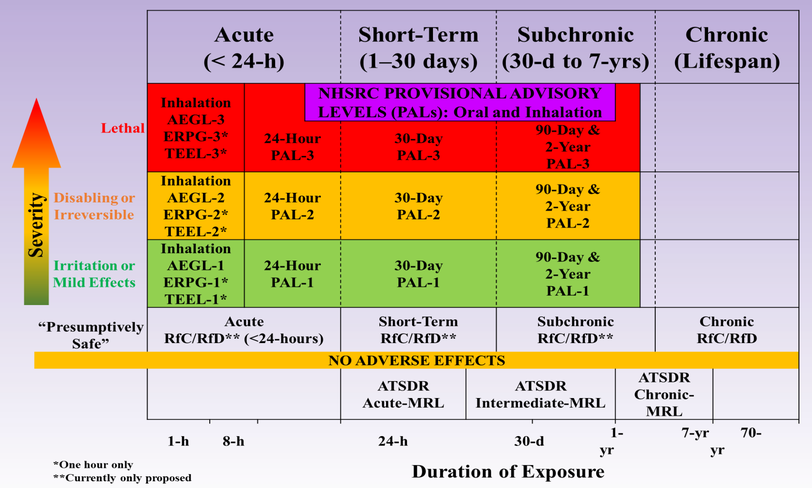
- Toxic Release Inventory.
- High Production Volume chemicals list.
- Extremely Hazardous Substances list.
- Chemical Terrorism Risk Assessment database.
- Release inventories maintained by the U.S. Coast Guard, Department of Transportation and Centers for Disease Control and Prevention.

ESCALATION OF ACRYLONITRILE EFFECTS

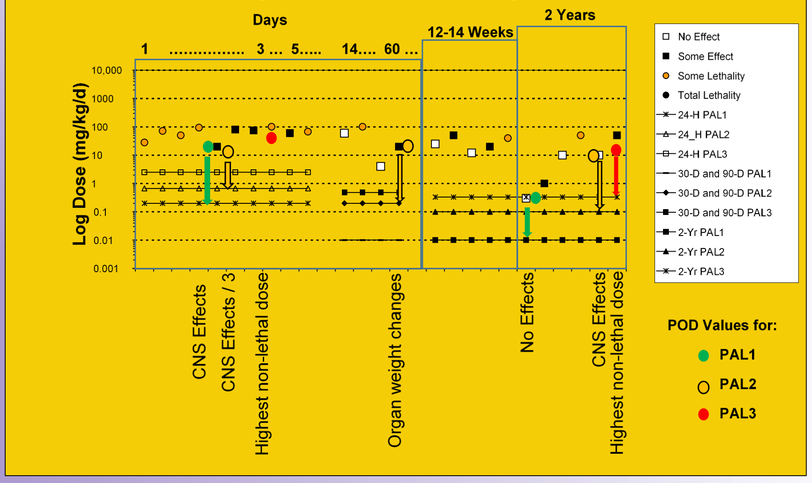


POINT OF DEPARTURE AND RISK VALUES





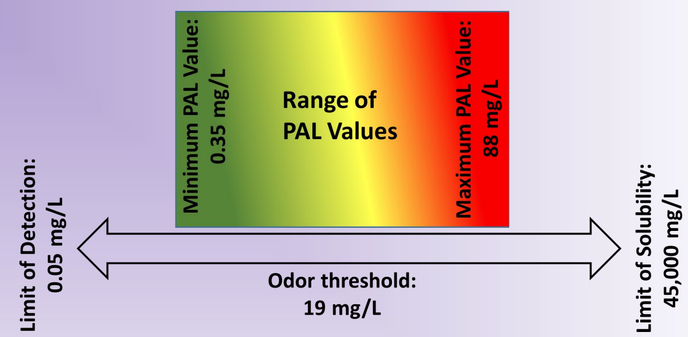
Acrylonitrile Oral Effects by Duration



PROVISIONAL ADVISORY LEVELS (PALs) for ACRYLONITRILE IN WATER (mg/L)

Guideline	Exposure Duration			
	24 Hours	30 Days	90 days	2 Years
PAL1	7	0.35	0.35	0.35
PAL2	23	7	7	3.5
PAL3	88	17	17	12

NOTABLE ACRYLONITRILE CONCENTRATIONS IN WATER



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Young RA et al. (2009). Overview of the standing operating procedures for the development of provisional advisory levels. *Inhalation Toxicology* 21 (Supl 3): 1-11.

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Underground Transport Restoration Program
Lab to field studies by EPA researchers

Overview

The Department of Homeland Security (DHS) Underground Transport Restoration (UTR) Program is working with EPA and other agencies to identify and evaluate methods for addressing a wide area contamination incident involving an underground transit system, investigating both physical structures (tunnels and stations) as well as rolling stock (railcars). This project addresses a high-priority need to improve recovery capabilities in the event of a biological release. Scientists and engineers at EPA are contributing to the project by conducting experiments to evaluate the efficacy of various decontaminants and procedures for safe, efficient, and cost-friendly remediation of a subway system which has been contaminated by a biological agent.

Lab Studies

Volumetric Decontamination Studies

Methyl Bromide (MB) Fumigation

EPA examined the efficacy of MB for the decontamination of indoor and outdoor materials contaminated with *B. anthracis* spores. Results of this study revealed the influence of relative humidity during fumigation, where greater efficacy was achieved at higher relative humidity.

Chlorine Dioxide (ClO_2) Fumigation

Researchers evaluated ClO_2's ability to decontaminate in diverse environmental conditions on sample materials with varying levels of surface grime obtained from a subway system. Both higher temperatures and relative humidity created the best conditions for effective inactivation of spores.

Fogging Technology & Sporicidal Decontamination

Tests were conducted in pilot-scale chambers to assess the efficacy of different foggers and sporicidal liquids to inactivate *B. anthracis* and surrogate spores on subway system- and railcar-related materials.

Emergent Composite Sampling Methods

Robotic floor cleaners, commercial wet vacuums, and aggressive air sampling are three emerging composite sampling methods being evaluated at EPA for determining residual environmental contamination levels of biological agents. These alternative methods provide the potential to require fewer samples resulting in reduced analytical burden and lowered labor needs and risks than traditional surface sampling methods.

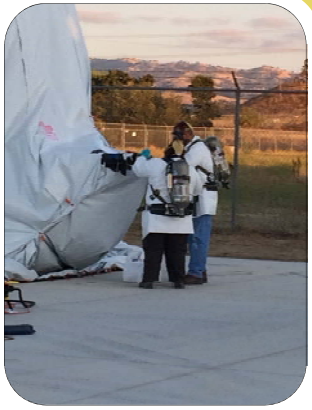
Commercial Equipment Survey & Evaluation

One of the goals of the UTR Program is to determine what already-available equipment can be implemented for rapid decontamination following a biological terror incident. The Commercial Equipment Survey and Evaluation identified and tested sprayers and foggers which could be used to decontaminate subway infrastructure (tunnels and platforms) following such an event.

Field Studies

Subway Railcar Decontamination

In July 2015, a field-test was conducted to evaluate the efficacy of MB as a decontaminant and the conditions necessary for its use in a real-world biological attack.



Samples of various materials from a subway railcar contaminated with *B. anthracis* Sterne strain spores were placed in a railcar which was subsequently fumigated with MB then aerated.

From this study, certain operational conclusions were revealed resulting in recommendations for time, temperature, relative humidity, and fumigant concentration conditions in the event of a contamination incident.

Operational Technology Demonstration

In Fall 2016, EPA researchers and responders participated in an interagency effort to demonstrate technologies resulting from these lab studies in a mock subway system located at Fort A.P. Hill, VA. The goal of this project is to apply and evaluate field-level mass transportation biological remediation on *Bacillus atrophaeus*, a *B. anthracis* surrogate.

Two cycles of release and decontamination occurred, with necessary sampling in between. The first phase utilized diluted bleach dispersed by a fogging system technology, and the second phase used a commercial pressure sprayer deploying a pH adjusted bleach solution. Each round incorporated a cost analysis of the decontamination method as well as subsequent efficacy results. Secondary studies included an aerosol knock down barrier evaluation, a gel application study, and an aggressive air sampling study.

Disclaimer

The photographs included are for illustration purposes. EPA does not approve or endorse the products or their manufacturers. EPA does not endorse the purchase or sale of any commercial products or services.

Contact

Lukas Oudejans oudejans.lukas@epa.gov or Shannon Serre serre.shannon@epa.gov

Boron Doped Diamond Electrochemical Advanced Oxidative Process Treatment of Heavily Contaminated Water for Drain Disposal and POTW Acceptance

- Rebecca Phillips, Oak Ridge Institute for Science and Education Research Program
- Ryan James, Battelle • Matthew Magnuson, U.S. EPA Homeland Security Research Program (HSRP)

United States Environmental Protection Agency

Objectives

The EPA HSRP is investigating the use of a boron doped diamond electrode (BDDE) advanced oxidation process (AOP), along with UV/H_2O_2 and O_3/H_2O_2 AOPs, as potential strategies to treat organic contaminants in water. This contamination could be introduced via intentional or unintentional incidents or through decontamination response activities to such incidents.

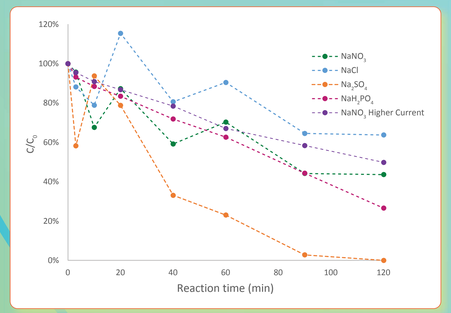
Materials and Methods

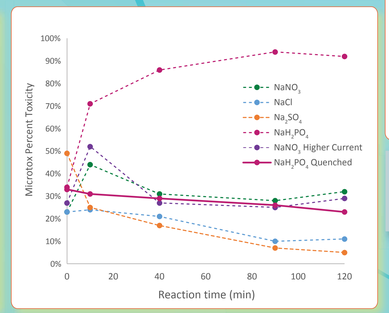
A BDDE cell, operated at either 4.4 or 8.8 amps, was used to treat 10L of a 0.05N electrolyte and 10ppm contaminant solution. The electrodes were coated with thin film diamonds (Advanced Diamond Technologies, Romeoville, IL), allowing the use of higher currents and the production of hydroxyl radicals on the electrode surface. Electrolytes included NaCl, $NaNO_3$, Na_2PO_4, and Na_2SO_4. Propanil, an herbicide, is utilized as a model contaminant. Treated water was returned to the supply reservoir for mixing and further treatment. Samples were analyzed for pH, contaminant degradation using LC-MS/MS, and the Microtox toxicity assay. Samples for certain trials were also analyzed for total organic carbon. Trials ran for 2, 8, 24, or 72 hours with samples drawn at predetermined intervals throughout the experiment. DPD and DPD/peroxidase tests, designed to detect chlorine and hydrogen peroxide, were performed using a Hach handheld colorimeter to indicate oxidant presence, as well as to verify oxidant quenching using sodium thiosulfate with certain electrolytes.

Why BDDE?

The BDDE process exhibits several advantages over other electrodes and AOPs that make it an excellent candidate as an AOP treatment. BDDEs are able to attain a higher range of working potentials and are more stable than other types of electrodes, allowing them to achieve higher performance compared to other electrode materials. This higher performance results in the production of hydroxyl radicals, as well as other mixed oxidants. The BDDE AOP may also be operated without reagents, unlike many other AOPs, thereby reducing design and maintenance considerations.

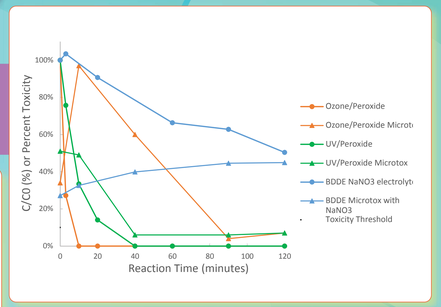
Electrolyte Effect

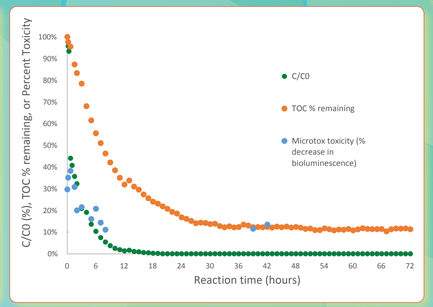




The use of different electrolytes may yield varying degrees of contaminant (propanil) degradation (left panel) and resulting toxicity (right panel). The electrolytes themselves may also contribute to the toxicity, indicated by the higher toxicities for electrolytes such as $NaNO_3$ vs. NaCl in spite of greater contaminant destruction. Ease of use was another factor in electrolyte choice and included consideration of potential byproduct formation, in particular for NaCl, as well as pH adjustment issues, in particular for NaH_2PO_4, which has significant buffering capacity. In light of these considerations, the $NaNO_3$ was selected for further trials.

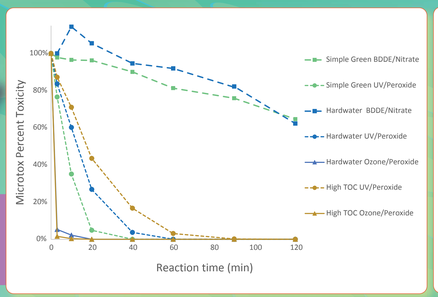
AOP Comparison

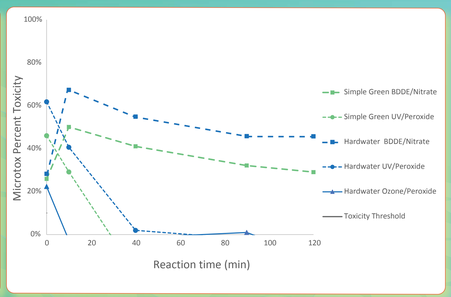




The three different AOPs yield different degrees of contaminant reduction during the two hour treatment time, as well as different microtox toxicity profiles (left panel). The O_3/H_2O_2 AOP yields the fastest contaminant degradation, while the BDDE yields the slowest. Given a longer treatment time, the BDDE system demonstrates that both the TOC and the microtox toxicity decline as propanil is degraded, eventually reaching complete propanil removal and toxicity reduction below the toxicity threshold (right panel). The decrease in TOC could result either from mineralization and offgassing of CO_2 or from the transformation of propanil into volatile reaction intermediates.

Matrix Effects





Hard water, soapy (0.01% Simple Green) and higher TOC (9 mg/L) background matrices yielded slower contaminant degradation compared with a dechlorinated tap water matrix (left panel). Contaminant degradation remained fastest, for all matrices, in the O_3/H_2O_2 AOP and slowest for all matrices in the BDDE system. Unlike the dechlorinated drinking water samples, increases in microbial toxicity during treatment time were only found in the BDDE AOP for the alternative matrices and were followed by a subsequent decrease in microbial toxicity (right panel). Microbial toxicity for the hard water and soapy water in the other two AOPs decreased to below the toxicity threshold before the end of the 2 hour treatment time.

Results

1) This study examines the efficacy of BDDE treatment for a model contaminant at varying current densities, reaction times, electrolyte compositions, and background matrices. Two current densities, four reaction times, four electrolytes, and four background matrices were tested.
2) Contaminant degradation is measured at pre-determined time points throughout each reaction using LC-MS/MS. All conditions tested yielded some degree of contaminant degradation, however reaction conditions, such as reaction times, electrolyte composition, and background matrices, did affect the extent of that contaminant degradation.
3) Contaminant degradation results are coupled with microbial toxicity results to indicate AOP performance. Microbial toxicity is rarely reported for BDDE processes. In this study, microbial toxicity is indicated by the Microtox toxicity assay, which simulates toxicity to receiving waters.
4) Microbial toxicity, along with contaminant degradation, varied with different electrolytes, reaction times, and background matrixes. Longer reaction times yielded reductions in toxicity to below the toxicity threshold for the Microtox assay. Oxidant quenching was also found to affect microbial toxicity measurements.
5) The BDDE performance is also compared with that of more traditional O_3/H_2O_2 and UV/H_2O_2 AOP technologies. The BDDE system generally achieves slower contaminant degradation and toxicity reductions compared to the other AOPs.

Conclusions

1) The BDDE system likely produces mixed oxidants alongside hydroxyl radicals. The identity of the mixed oxidants varies with each electrolyte; for instance, chloride may produce hypochlorite, and sulfate may produce persulfate. These mixed oxidants may result in different rates of contaminant treatment, resulting toxicities, and potential byproduct formation.
2) Given enough treatment time, the BDDE system may achieve similar contaminant and toxicity reductions to those of the other AOPs, including complete degradation of propanil and reduction in the overall microbial toxicity. Further, significant reductions in TOC were observed using the BDDE system, indicating that some degree of mineralization may have been achieved.
3) Contaminant degradation and microbial toxicity reductions are observed in both clean waters and in alternative matrices, including hard water, higher TOC water, and soapy water. As may be expected, degradation was slower in the presence of the alternative matrices, likely due to competition for oxidants.
4) In certain applications, the simplicity and lack of potentially problematic reagents may make implementation of the BDDE system preferable to that of other AOP approaches.

The U.S. Environmental Protection Agency through its Office of Research and Development funded and collaborated in the research described here under Contract EP-C-10-001. It has been subjected to the Agency's review and has been approved for public presentation. EPA does not endorse the purchase or sale of any commercial products or services. This project was supported in part by an appointment to the Research Participation Program at US EPA, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and EPA.

National Institute of Environmental Health Sciences

Jim Remington, RN, National Institute of Environmental Health Sciences, Worker Training Program (WTP)
Joy Lee Pearson, National Clearinghouse for Worker Safety and Health Training
Nina Jaitly, MD, MPH, CPH, National Institute of Environmental Health Sciences, WTP

NIEHS WTP Framework for Implementing A National Biosafety and Infectious Disease Response Training (IDRT) Program

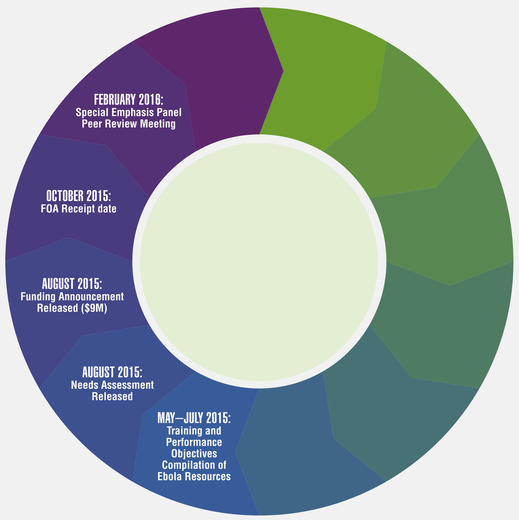
Background/Significance

In an era of emerging and re-emerging communicable disease health threats, the importance of infection prevention and control (IPC) measures in healthcare settings should not be underestimated. Transmission of communicable disease and pathogens is an ever-evolving subject, and the risk of transmission of pathogens outside of healthcare settings is no exception. The recent cases of Ebola virus disease (EVD) in the United States (U.S.) remind us that infectious disease threats at both the community and national levels continue to challenge the capacity of health systems, occupational settings, and communities to adequately protect people from exposure to microorganisms (e.g., bacteria, viruses, and spores) that may cause illness or infection.

During the response to the EVD Biosafety pandemic in 2014, NIEHS WTP leveraged existing awardees to provide domestic EVD preparedness training to more than 7,000 workers in 18 states. Through train-the-trainer (TTT), awareness- and operations-level instruction, the supplemental awards focused on the inclusion of hazard recognition, mitigation, and prevention of potential EVD exposures of healthcare and non-healthcare workers. Following efforts to build an intra-agency coalition and workshop to discuss priorities, identify high-risk populations, and need for curricula development and training, in early 2015, NIEHS received $10 million to develop biosafety training programs for U.S. based workers and the NIEHS WTP was charged to lead future efforts over three years (2016-2019).

Objectives:

We will highlight 1) the process used to elicit grant applications and, 2) promising approaches and practice for education in IPC to train approximately 35, 000 workers across the U.S., specifically, 23 high-risk occupational target populations. Additionally, unique collaborations that empower national capacity for ongoing IDRT is also presented.



Methods

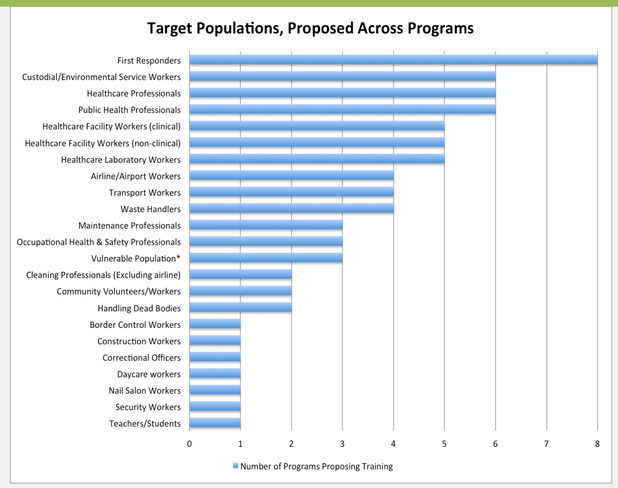
A review of 8 awarded programs (awardees) in response to the NIEHS WTP Ebola Biosafety and IDRT UH4 Program was conducted. Proposed target populations, training strategies, and collaborations for national capacity were grouped into common themes as proposed.

Results/Analysis:

As part of a national coordinated effort to prevent, control and respond to potential occupational exposure to organisms causing infectious diseases, through cooperative agreement mechanism, awardees will:

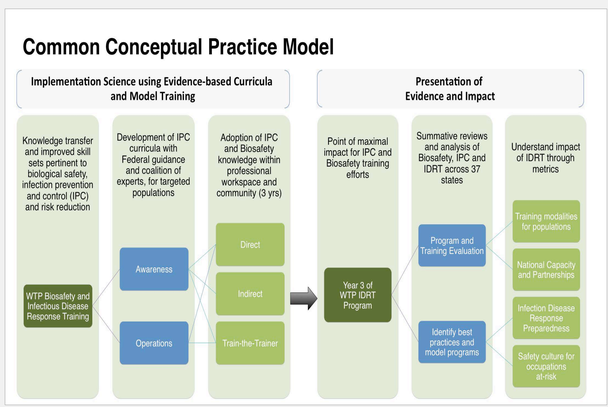
- Develop curriculum for workers who are at high risk of coming in contact with EVD and other emerging infectious diseases, through potentially contaminated materials (i.e., biological, chemical or radiological) or infected individuals
- Integrate collaborators from infectious disease (ID), occupational medicine, industrial hygiene, public health, and other worker safety fields
- Embrace an all-hazards approach within the whole-of-community
- Include standards and guidance documents as suggested by NIEHS federal and state partners
- Focus primarily on awareness and operations level training and TTT modalities
- Integrate new or existing partnerships to impart strong risk assessment and IPC skills for workers at-risk in various job sectors
- Together, consortia members will build national capacity using a common practice model

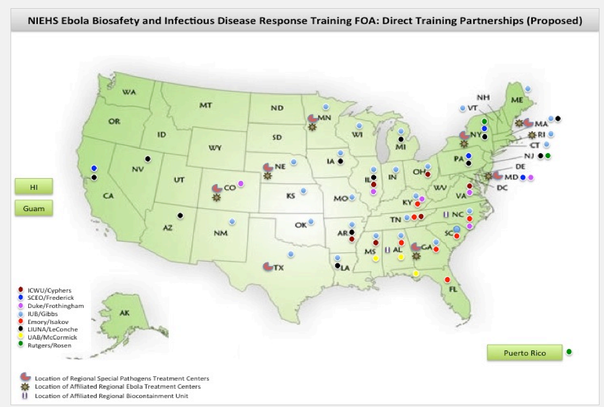
Results/Analysis Continued:



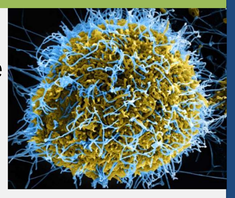
Target Population Category by Applicant

Population Category	ICWU	SCEO	DUKE	IUB	EMORY	LIUNA	UAB	RUTGERS
	Cyphers	*Frederick*	*Frothingham*	*Gibbs*	*Isakov*	*LeConche*	*McCormack*	*Rosen*
Airline/Airport Workers	X	X	X			X		
Border Control Workers		X	X					
Cleaning Professional (excluding airline)		X				X		
Community Volunteers/Workers		X			X			X
Construction Workers						X		
Correctional Officers	X							
Custodial/Environmental Service Workers	X		X	X	X	X	X	
Daycare Workers	X							
First Responders	X	X	X	X	X	X	X	X
Handling Dead Bodies		X		X				
Healthcare Facility Workers (clinical)	X	X	X		X		X	
Healthcare Facility Workers (non-clinical)		X	X		X	X		X
Healthcare Laboratory Workers	X	X	X		X		X	
Healthcare Professionals	X	X	X		X		X	X
Maintenance Professional	X	X						X
Nail Salon Workers								X
Public Health Professionals	X		X		X			X
Occupational Health & Safety Activists	X			X	X	X		X
Security Workers			X					
Teachers/Students	X							
Transport Workers		X	X	X				X
Vulnerable Populations			X	X		X		X
Waste Handlers		X	X	X			X	
Total high risk categories, grouped	12	13	13	7	9	8	6	10





Discussion



The NIEHS WTP is at a groundbreaking point where professionals in disaster response, hazardous waste management, infection control, biosafety training, medicine, occupational health and safety, and public health are collectively bridging the science of IPC and expanding biosafety concepts within occupational worker safety practices. It is evident that this effort cannot be successful without addressing some of the invisible silos, training gaps in the respective fields, and semantic heterogeneity associated with the IPC sector. Over the next three years, the NIEHS WTP awardees will disseminate environmental infection control and hazard recognition training modules, using awareness, operation and TTT, within a large array of occupational and community settings in healthcare and non-healthcare job sectors. A novel approach to use hazard identification, risk characterization, and risk assessment will support the build out of improved hazard communication, stratification and critical judgment skills that are needed to handle the broader context of infectious disease outbreaks. Curricula will have outcomes that demonstrate: (a) increased capacity to provide IPC biosafety training that addresses population-sepcific worker safety and health protection concerns, and (b) improved knowledge and skills for workers with potential exposure to contaminated materials or infected individuals through their job duties. Implementation using a common conceptual practice model will assure that evidence-based practices are disseminated. Awareness and operation training approaches will empower workers to advocate for safety practices and resources for building of, or amending, ID exposure control plans. TTT approaches will help build a cadre of master trainers who will facilitate with build out of advanced training nationwide. These efforts hold the potential to promote synergy, identify best practices and successful training strategies for IPC in varied occupational settings or populations. It may further facilitate approaches that may systematize broader, national or even standardized IDRT framework for infectious diseases outbreaks as they emerge in both healthcare and non-healthcare settings in the U.S. in the future.

Employing microbiological surrogates to compare chlorine dioxide fumigation and heat treatment of commercial poultry barns under field conditions

Julian N. Rosenberg[1,*], Douwe F. Mason[1], M. Worth Calfee[2], Eric R. Rhodes[2], Shannon D. Serre[3], Madeline C. Bette[1], Shawn P. Ryan[2], John Y. Mason[1]

[1] BioWALL LLC, a Sabre Company, 1891 New Scotland Road, Slingerlands, NY 12159
[2] U.S. EPA, National Homeland Security Research Center, Office of Research and Development
[*] To whom correspondence should be addressed: jrosenberg@sabrecompanies.com
[3] U.S. EPA, CBRN Consequence Management & Advisory Division, Office of Land and Emergency Management

Overview

Commercial-scale livestock production

facilities contaminated with highly pathogenic avian influenza (HPAI) or other biological contaminants pose potential risks to human and animal health following an outbreak. Current procedures for decontaminating viruses and bacteria in complex agricultural facilities are limited. Further, there is a need for rapid facility-clearance methods for all pathogens in order to return livestock operations to pre-incident risk levels. Under a cooperative research and development agreement with the EPA, the virucidal and sporicidal efficacies of chlorine dioxide (ClO_2) fumigation were compared to heat treatment of egg layer barns in a large-scale field test. A test matrix of biological surrogates and material coupons was devised to compare pathogen inactivation on diverse surfaces under challenging fall/winter conditions.

Research goals:

① **Directly compare the efficacy of two decontamination methods** used for commercial poultry barns: **(1)** chlorine dioxide fumigation targeting 25,000 ppm_v-hrs at 75˚F and 85% RH and **(2)** heat treatment following USDA guidelines of 100-120˚F for seven days with at least three consecutive days ≥100˚F.

② **Develop & evaluate mixed panel of non-pathogenic surrogates** for viral and bacterial spore contaminants common to the poultry industry and more broadly relevant to public health.

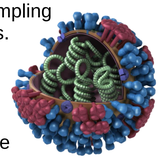
③ **Examine material effects of each decontamination process** on visible surfaces and assess structural integrity of each barn following the specified treatment conditions.

④ **Analyze efficacy results with respect to stakeholder needs** including biosecurity implications, cost considerations, and operational feasibility.

"Sentinel" Surrogate Approach

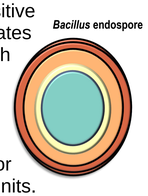
Clearing a barn for repopulation

using currently accepted methods involves physically swabbing representative surfaces of the barn to collect environmental samples, which are then subjected to PCR analysis and/or viral isolation. Surface sampling by swabbing is known to be unreliable, especially on porous surfaces. Moreover, viral isolation takes weeks to process and can overload laboratories during outbreak events. The rationale for the surrogate approach stems from the need to rapidly determine disinfection efficacy on actual building materials in order to efficiently clear contaminated buildings with a high degree of confidence. As such, the biological surrogates employed must be representative of the target pathogens, possess similar resistance to disinfectants as those pathogens, and have bioassays that can deliver results with relatively short timeframes.



Influenza virus
worldofviruses.unl.edu/category/image-wall/inside-viruses/

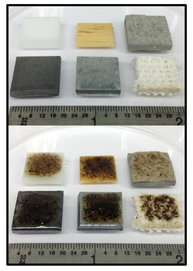
For these reasons, MS2 bacteriophage (ATCC 15597-B1, non-enveloped viral surrogate) and *Bacillus subtilis* endospores (ATCC 6633, gram-positive bacteria) served as surrogates for the field test. These "sentinel" surrogates (depicted to the right) were inoculated on various material coupons, each prepared in triplicate, with and without organic soil loading, then sealed individually in Tyvek/Tyvek pouches to prevent cross-contamination. In total, over 1,000 coupons were deployed for this field test, including relevant controls. Following the respective treatments, each set of biological indicators was retrieved and sent to third party contract labs for whole coupon extraction and enumeration of plaque or colony forming units.

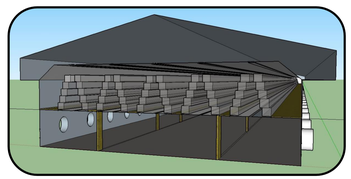


Diverse Material Matrices

Porous and non-porous materials

representing the varied surfaces and structural components present in poultry barns were produced as 0.25 in^2 reference coupons and inoculated with either MS2 phage or *B. subtilis* spores. The suite of reference materials included unpainted pine plywood, cotton belt, concrete, black iron, galvanized steel, and high-density polyethylene. In order to simulate the actual soiling loads found in working barns, both clean and soiled coupons were used in the present study (right). Representative organic dust comprised of fresh poultry manure was sourced from a commercial chicken farm. The manure was desiccated, mechanically pulverized, and applied to each coupon as a base grime layer in a 10% solution of eicosane ($C_{20}H_{42}$) as a carrier.





Spatially distributed real-time monitoring of both test barns

during the field test enabled off-site participants to view respective treatment conditions via data link. In addition to EPA and USDA collaborators, project observers included senior officers of the National Guard, FEMA, U.S. Air Force, and Homeland Security. The live data feed relayed temperature, relative humidity, and ClO_2 concentration (as applicable) from 10 locations in each of the test barns (left). "Sentinel" surrogates were distributed at 5 of the 10 locations in each barn. Commercial spore strips were staged at all 20 locations.

The U.S. Environmental Protection Agency, through its Office of Research and Development, collaborated in the research described here under a CRADA with SABRE Corp. It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.

Comparative Virucidal & Sporicidal Efficacies

Viable surrogate populations recovered

from clean and soiled coupons revealed anticipated trends in log-reductions, chiefly organic load negatively impacting the efficacy of each treatment while also improving surrogate recovery. However, the field test also shed light on some intriguing data regarding viral persistence. As illustrated in the plots to the right, white bars represent average CFU/PFU recovered from control coupons; gray bars show the average CFU/PFU collected from treated coupons (five locations, each in triplicate). Taken together, the results demonstrate pervasive sporicidal sterilization on the clean *B. subtilis* spore coupons following ClO_2 fumigation (**Fig. 1a**) and near 6-log reductions on the soiled set (**Fig. 1b**). Corresponding coupons subjected to heat treatment did not exhibit any significant reductions in viable spores (**Fig. 1c-d**).

As for the viral surrogate, MS2 populations recovered from coupons fumigated with ClO_2 demonstrated widespread virucidal efficacy (>>3-log reductions) on all chlorine dioxide coupons (**Fig. 2a-b**). Alternatively, none of the MS2 coupons treated in the heat barn exhibited >3-log reductions in PFU (**Fig. 2c-d**). In fact, viable MS2 was detected in greater abundance on some heat treated coupons for which poor phage stability was observed on the respective controls (iron, steel, HDPE, and concrete), suggesting that heat treatment at low humidity may augment the persistence of non-enveloped MS2 phage on these surfaces relative to winter conditions.

Figure 1. *Bacillus* Spore Recovery

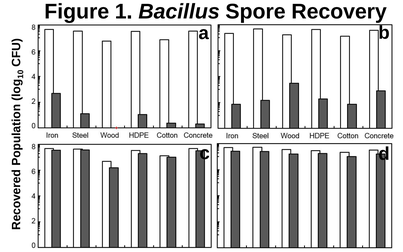


Fig. 1. All heat and ClO_2 controls were maintained at 10˚C ± 10˚C with 60% ± 10% RH and 23˚C ± 10˚C with 75% ± 10% RH, respectively. ☐ Control ■ Treated

Figure 2. MS2 Phage Recovery

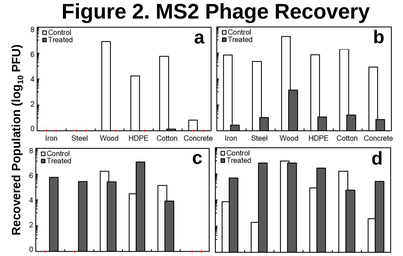


Fig. 2. In some cases, control and/or treated coupons were below the limit of detection for the plaque assay (+), indicating complete inactivation of phage.

Future Work & Broader Impacts

Valuable information for stakeholders

in the poultry industry was generated and disseminated under this CRADA. Although the current study focused on barns affected by HPAI, these methodologies examined should be relevant to the protein production industry as a whole. The use of surrogate microorganisms that represent far more robust pathogens than an enveloped influenza virus (*i.e.*, spores) generated working data for more broad decontamination preparedness. Large-scale sterilization technology will play a major role in the future of agricultural outbreak response—providing reliable and rapid decontamination, mitigating further spread of disease, and enabling producers to return to production as soon as possible, thus dampening extended economic impacts.

The first head-to-head comparison

of ClO_2 and heat treatment under field conditions offers a robust data set of quantified virucidal and sporicidal efficacy on half a dozen high-challenge surfaces, similar to those encountered in egg layer barns. The US HPAI outbreak of summer 2015 resulted in the death of 50 million chickens and turkeys, billions of dollars in taxpayer expenditures, and hundreds of millions of dollars in producer losses and downtime. However, there are limited data confirming that disinfection processes used in the 2015 response were efficacious. From an analytical standpoint, the turn-around time for the surrogate-based approach piloted during this project requires days, instead of weeks, and is far more reliable than environmental swabbing. We hope that the results of this work provide sound scientific evidence for future decisions related to choice of remediation options, ultimately allowing producers to confidently return to production in much shorter timeframes. Follow-up benchtop studies will be pursued under laboratory conditions to further compare surrogate-pathogen equivalency and potentially broaden the panel of surrogates.

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Fate and Transport of VX and Sulfur Mustard across a Permeable Layer into Porous Subsurfaces

Lukas Oudejans[1], David See[2], Daniel Chappie[2], Anthony Ellingson[2], and Katherine Mitchell[2].

[1] EPA NHSRC, Research Triangle Park, NC; [2] Battelle, Columbus, OH.

Lukas Oudejans | Oudejans.Lukas@epa.gov | 919-541-2973

Abstract

A release of a chemical warfare agent (CWA) into the urban environment has the potential to contaminate painted and/or sealed building materials that would require remediation. This study investigated the fate and transport of two CWAs, VX and sulfur mustard (HD), deposited on painted/sealed stainless steel and on freestanding paint/sealant films. In the case of freestanding films, a solid phase extraction (SPE) disk was placed under the test coupon to mimic a porous material and to collect agent which permeated through the paint/sealant films. Three types of paints and two types of sealants were evaluated. Samples were challenged with 2 microliters of VX or HD and then held at ambient laboratory conditions for durations ranging from 3 hours to 48 hours (HD) or 72 hours (VX). Following contact of the agent with the surface, residual CWA was measured via wipe-sampling of the coupon surface, solvent extraction of the steel or freestanding film, and solvent extraction of the underlying SPE. Extracts were then analyzed for VX or HD using gas chromatography/mass spectrometry. CWA recoveries from the film wipe, film extraction, and SPE extraction were measured and reported, as well as the total CWA recovery based on the summation of the individual recoveries.

Generally, total VX recoveries and film wipe recoveries decreased with increasing weathering (≤62% after 72 hours and ≤19% after 72 hours, respectively). VX was generally detected from the film extractions (4.4% to 47% after 72 hours) and SPE extractions (<2.5% to 19% after 72 hours), but consistently increasing or decreasing trends were not apparent across all the paints/sealants. HD recoveries from surface wiping rapidly decreased (≤2.0% after 48 hours), but HD remained recoverable from film and SPE extractions. On painted surfaces, total HD recoveries remained high (≥72%) after 48 hours, except in the case of oil gloss painted stainless steel (25%). Total HD recoveries obtained during sealant testing tended to be lower (generally ≤48% after 48 hours), with the exception of polyurethane sealant film placed over SPE disk, which yielded a total recovery of 86% after 48 hours (73% of which came from the underlying SPE disk).

This research clearly demonstrates that VX and HD can penetrate through paints/sealants and are quite capable of migrating into underlying porous materials. Surface sampling may capture only a fraction of the VX and HD retained in paint/sealant layers and/or underlying porous materials, thus sampling and remediation strategies must address the potential for CWA to be retained within porous materials beneath painted/sealed surfaces.

Methods

Testing was conducted with the following paints and sealants:

- Latex flat, latex semi-gloss, and oil gloss paint
- Epoxy and polyurethane sealant

The paints/sealants were applied to stainless steel or made into freestanding (FS) films that were placed on top of a porous material, i.e., SPE disk.

Two microliters of VX or HD were spiked onto the paint/sealant surfaces. Figure 1 shows HD applied to sealed stainless steel and Figure 2 shows a FS paint film being removed from an SPE disk (after the FS paint film was wipe-sampled).

Figure 1. HD applied to sealed steel (white is epoxy, grey is polyurethane).

Figure 2. Removal of FS paint film from SPE disk.

Methods (continued)

A Low Volatility Agent Permeation (LVAP) setup was used for testing with the SPE disk. The setup used a latex gasket and weighted washer to ensure close contact between the FS film (5.0 cm diameter) and SPE disk, while also inhibiting fugitive CWA vapor from reaching the SPE disk. This ensured that CWA recovered from the SPE disk was attributed to permeation rather than vapor adsorption. The SPE disk represents the porous material. A photograph of the LVAP setup is shown in Figure 3 and a diagram is provided in Figure 4.

Figure 3. LVAP photograph.

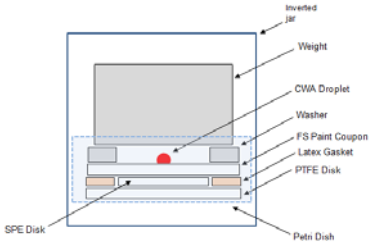


Figure 4. LVAP diagram.

The CWA challenged materials were allowed to weather under ambient laboratory conditions (18-23 °C; 28-49% RH across all tests) for pre-determined times, ranging from 3 to 72 hours.

Residual CWA was then measured via:

- wipe-sampling of the painted/sealed surface (wipe),
- solvent extraction of the stainless steel or FS film post-wipe sampling (coupon), and
- solvent extraction of the underlying SPE disk (SPE).

2"x2" 4-ply rayon/polyester (gauze) wipes were used as the wipe-sampling media. Hexane was used as the extraction solvent and was used to wet the gauze during wipe sampling. Samples were extracted by sonication for 10 minutes.

Extracts were analyzed for CWA using gas chromatography/mass spectrometry. The VX and HD residual mass detection limit was 2.6 µg for wipe samples and 2.5 µg for the other samples.

Results

CWA recoveries are presented in Figures 5a/5b for VX and 6a/6b for HD. Figure 7 shows the curve fitting of HD recovery associated with the FS latex semi-gloss paint film test. The curves were fitted using first order equations that simultaneously accounted for the rate of evaporation, absorption into the paint, and breakthrough from the paint into the underlying SPE disk.

In general:

- CWA was recovered from most wipes, coupons, and SPE disks.
- CWA recoveries from wipes generally decreased over time.
- CWA recoveries from SPE disks generally increased over time.

For VX:

- VX appeared to have a greater affinity (higher %VX recoveries from coupons) for latex semi-gloss paint than the other paints tested.
- VX recoveries from wipe samples tended to be higher than those for HD.
- Total VX recoveries decreased faster than total HD recoveries, except when associated with epoxy sealed steel (steel epoxy) and FS epoxy film (SPE epoxy).

For HD:

- HD tended to have a greater affinity (higher %HD recoveries) for the paints/sealants (coupons) and SPE disk than VX, except for epoxy sealed materials.
- HD recoveries from wipe sampling rapidly decreased to ≤2% after 48 hours.
- Total HD recoveries from the painted surfaces and the polyurethane sealed surfaces were greater than the VX recoveries.

Results (continued)

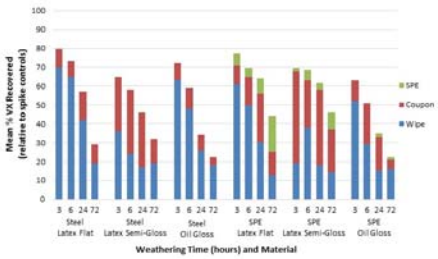


Figure 5a. VX recovery from paint tests.

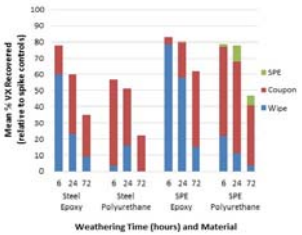


Figure 5b. VX recovery from sealant tests.

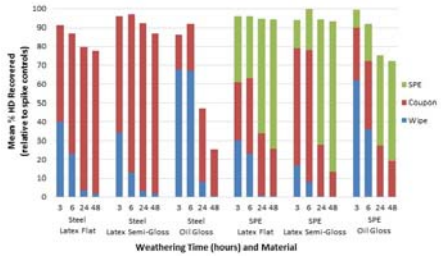


Figure 6a. HD recovery from paint tests.

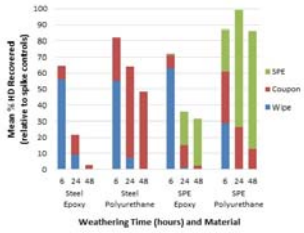


Figure 6b. HD recovery from sealant tests.

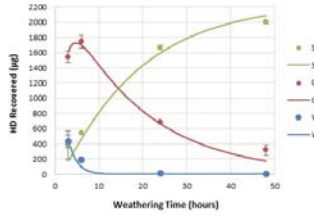


Figure 7 (left). Scatter plot with fitted curve of HD recovery from FS latex semi-gloss paint.

- Wipe represents non-evaporated HD.
- Coupon represents HD absorbed into paint.
- SPE represents HD breakthrough.

The curve fit (R^2=0.983) was much better than for other combinations of VX/HD and paint/sealant.

Error bars represent ± one standard deviation.

Conclusions

- VX and HD can penetrate into paints/sealants and migrate into underlying porous materials.
- Permeation is influenced by the CWA and paint/sealant and time dependent.
- Porous materials might serve as reservoirs for CWA increasing persistence.
- Wipe sampling may capture only a fraction of the CWA retained.
- Sampling and remediation strategies must account for CWA permeation to materials.

Disclaimer

The U.S. EPA, through its Office of Research and Development, funded and managed this investigation through Contract No. EP-C-11-038 Task Order 29 with Battelle. This document has been subjected to the Agency's review and has been approved for presentation. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names or commercial products, or services does not constitute EPA approval, endorsement or recommendation for use.

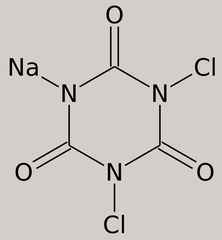
Gelled Formulations for Subway Decontamination

Mark D. Tucker, Patrick D. Burton, Matthew S. Tezak, Melissa R. Rosenthal and Zachary K. Meinelt

Sandia National Laboratories is a multi-mission laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000. SAND2016-10849C

Objective

Develop and deploy gelled decontamination formulations in the subway environment using readily available supplies.



- Sodium dichloroisocyanurate (DiChlor) is used as the oxidizing agent in swimming pools and drinking water sterilization.
- pH-amended bleach is used to minimize the corrosive effects of hypochlorite.
- 4% activated peroxide (modified Sandia DF-200)

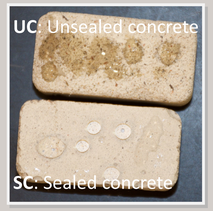
Increase contact time of decontaminant to:

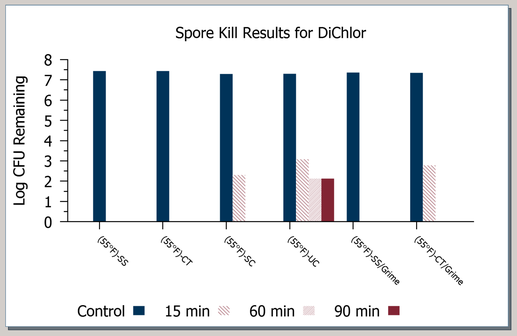
- Improve efficacy on vertical surfaces
- Improve efficacy at lower temperatures (55 °F)
- Minimize run-off to reduce waste generation

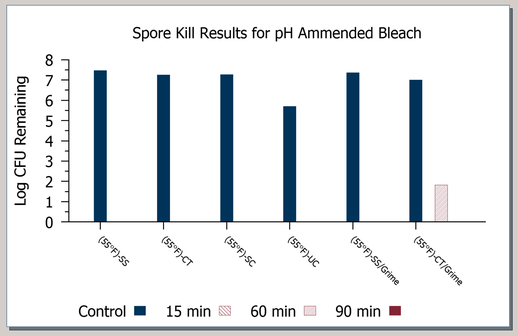
Development Test Results

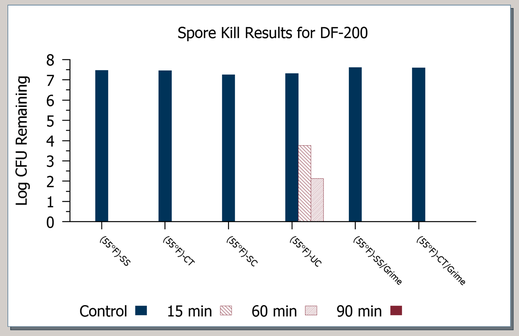
Spore kill efficacy of each decontaminant for *Bacillus atrophaeus* was evaluated under:

- Room (72 °F) & low (55 °F) temperature
- In-situ on test coupons
- With and without gelling material (Aerosil® 200 SiO_2)
- Contact times of 15, 60 and 90 minutes
- Surface contaminants (grime)









Underground Transport Restoration Project Testing at Operational Technology Demonstration

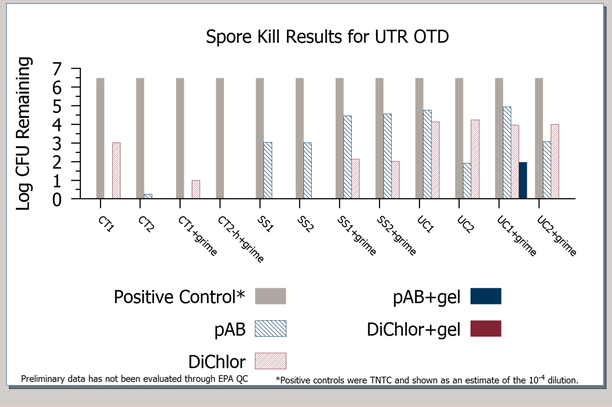
Test Conditions:

- Coupons were stored and tested at ambient (72 °F) temperature.
- Test material consisted of CT, SS and UC cut into 14"× 14" squares and deployed on the racks shown in Figure a.
- PAB and DiChlor with and without Aerosil® 200 (Figure b) were applied at an approximate coverage of 0.5 L/m^2 using a bleach-compatible garden sprayer (shown in Figure c).
- Sprayed coupons were allowed to rest for 60 minutes, then sampled.
- Unmodified and gelled tests were conducted in separate campaigns.


(a): Coupons were stored in sealed containers (left) at ambient temperature until deployment on the two racks


(b): 6 wt.% Aerosil 200


(c): Garden sprayers carried by testing team



Conclusions

Gelled decon solutions exhibited higher kill rates than unmodified liquid decontaminants applied directly to the test surfaces. A slight decrease in decon efficacy was observed for grime-coated coupons under laboratory conditions and the liquid (non-gelled) field test. Spores were only detected on a single plate (with low counts) for the gelled pAB; no counts were detected for the gelled DiChlor. This is likely due to the increased contact and residence time afforded by the viscous solutions.

Assessment of the use of Sodium Hydroxide for the destruction of ricin

N. Walker

CBR Division, Dstl, Porton Down, Salisbury, Wiltshire, SP4 0JQ.

Abstract

Ricin is a protein toxin found in the seeds of the castor oil plant *Ricinus communis*. It can be extracted from the seeds by a number of different methods producing an extract with high protein content, with ricin typically being 10 % of the total protein concentration in an aqueous extract. We are interested in methodologies for simple and rapid destruction of ricin.

Chlorine based decontaminants e.g. sodium or calcium hypochlorite, can be used to decontaminate protein toxins by denaturing and hydrolysing the toxin. A disadvantage of the addition of these chlorine based decontaminants to stocks of highly proteinaceous solutions is that they can cause frothing and the release of free chlorine resulting in a respiratory hazard to the operator.

We have therefore investigated the use of sodium hydroxide as a potential low technology, environmentally friendly and relatively safe method for the destruction of large quantities of aqueous ricin extract. Being a protein, NaOH will hydrolyse the amide bond between the amino acids of the ricin resulting in the toxin being broken down into small peptides and amino acids, thereby destroying the toxin. The efficiency of 10 %, 5 %, 2.5 % or 1 % w/v NaOH to hydrolyse ricin both in 500mM NaCl and in 500mM NaCl pH4 was assessed by an antibody based Hand Held Assay and SDS-PAGE.

Introduction

Ricin is a type II ribosome inactivating protein extracted from the seeds of the castor oil plant, *Ricinus communis*. It can be extracted using a number of different approaches. These include using solvents to remove the castor oil from the seeds prior to extraction of the toxin into an aqueous buffer [1] centrifugation to remove the castor oil from a seed homogenate in aqueous buffer [2] or using acetone to remove the castor oil producing a powdered product[3].

Whichever method is used a highly proteinaceous solution is produced with ricin being typically 10% of the total protein content.

A number of chemicals can be used to decontaminate protein toxins by denaturing and hydrolysing the toxin. Chlorine based decontaminants for example,sodium or calcium hypochlorite release free chlorine into solution which can off gas when added to solutions of high protein content. If being used to treat large volumes of proteinaceous material the chlorine released could pose a hazard to the operator and also leave large volumes of chlorinated waste to be disposed of.

NaOH although corrosive, does not off gas and once treatment has been proved effective can be neutralised by addition of an acid resulting generation of the acid's salt and water allowing the hydrolysate to be disposed of to drains.

We have investigated the use of 10 %, 5 %, 2.5 % or 1 % w/v NaOH to hydrolyse ricin in an aqueous extract with the efficiency of the hydrolysis being assessed by the antibody based Hand Held Assay (HHA) and SDS-PAGE.

Ricinus communis.

Ricinus communis seeds.

References

1. Olsnes S, Pihl A (1973) Different biological properties of the two constituent peptide chains of ricin, a toxic protein inhibiting protein synthesis. Biochemistry 12: 3121-3126.
2. Thullier P, Griffiths G (2009) Broad recognition of ricin toxins prepared from a range of Ricinus cultivars using immunochromatographic tests. Clin Toxicol (Phila) 47: 643-650.
3. Ovenden SP, Pigott EJ, Rochfort S, Bourne DJ (2014) Liquid Chromatography-Mass Spectrometry and Chemometric Analysis of Ricinus communis Extracts for Cultivar Identification. Phytochem Anal.

Materials and methods

Preparation of Ricin extract

100 g *Ricinus communis* seeds were blended in a Waring blender in 200 mL, 500mM NaCl. The blended seeds were then centrifuged at 18600 g for 30 minutes, the supernatant removed and centrifuged again at 27000 g for 20 minutes. The resultant extract was stored at -80 ºC until required.

Determination of total protein content

Total protein content was determined using the BCA protein assay (Pierce).

Determination of ricin content

The ricin content of the extract was determined by SDS-PAGE. Samples were run alongside a BSA standard curve on a 10-15% PhastGel™ (GE Healthcare), and stained using PhastGel Blue R™ (GE Healthcare). Bands were analysed using a GS-800 densitometer (Bio-Rad) and the Bio-Rad Quantity One software. Band densities of the BSA standards were plotted against concentration and using Linear Regression within GraphPad Prism the ricin concentration calculated from the density of the ricin band.

Small scale NaOH experiments

Aliquots of 800 µl extract were incubated with a final concentration of 10 %, 5 %, 2.5 % or 1 % NaOH w/v added from a 50 % NaOH w/v stock solution. Samples were taken at 30 minutes, 2, 4 and 24 hours and analysed by SDS-PAGE and Hand Held Assay.

Medium scale NaOH experiments

Aliquots of 100 mL extract were treated with a final concentration of 10 % NaOH w/v, added as NaOH pellets and stirred using a magnetic stirrer until the pellets had dissolved. Samples were taken at t=0, 30 minutes, 1, 2, 4 and 24 hours and analysed by SDS-PAGE and HHA. Four Litres of Bovine serum albumin (BSA) at 40 mg/mL in 500mM NaCl pH4 was treated with 10 % NaOH and stirred overhead. Samples were analysed by SDS-PAGE after 1 hour and 24 hours.

Hand Held Assay

Samples were diluted 1:100 in the assay buffer (150 mMNaCl, 10mM HEPES, 0.01 % Tween and 0.095 % sodium azide) provided with the Hand Held Assay (HHA) and 100 µl added to the test lane. The assay was read after 20 minutes.

SDS-PAGE

Samples were diluted 1:5 in water before mixing in a ratio of 2:1 of sample to Laemlli buffer containing no β-mercaptoethanol. Samples were run on a 10-15% PhastGel™ (GE Healthcare), and stained using PhastGel Blue R™ (GE Healthcare).

Results and Discussion

Prior to use both the HHA and SDS-PAGE running conditions were optimised for the presence of 10% NaOH. The crude ricin extract usedin this work had a total protein concentration of 40mg/mL and an estimated ricin concentration of 7mg/mL.

Small Scale Assessment

Initially 1mL small scale reactions were set up to assess the effect of NaOH on the ricin. From a 50 % stock solution, NaOH was added to the extract with or without the pH adjusted to pH 4 with acetic acid.

A concentration effect of NaOH was seen on the rate of hydrolysis of both the ricin and other proteins within the extract. In the HHA (Table 1.) which being antibody based detects both the presence, and structural integrity of the ricin, the Visual Score Card (VSC) score of the test line decreased at each time point with the ricin becoming undetectable after 4 hours at all NaOH concentrations.

The SDS-PAGE analysis (Figure 1.) complemented the HHA assay data. Within 30 minutes there was significant hydrolysis of the ricin and other proteins in the extract and by 24 hours in both the 5 % and 10 % NaOH reactions all protein was completely hydrolysed leaving a single band at the dye front of the gel.

	500mM NaCl adjusted to pH 4 % NaOH				500mM NaCl % NaOH				untreated control
	10%	5%	2.5 %	1 %	10%	5%	2.5 %	1 %	
t=30 min	5	6	7	8	5	6	8	9	10
t=2 hour	2	3	4	5	2	3	4	6	10
t=4 hour	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	10
t=24 hour	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	10

Table 1. Hand Held Assay VSC scores following the addition of varying concentrations of NaOH to ricin extract. The score is given in relation to the intensity of the test line in comparison with a VSC score card and ranges from 10, the highest, to 2 at which point the test line is only just visible.

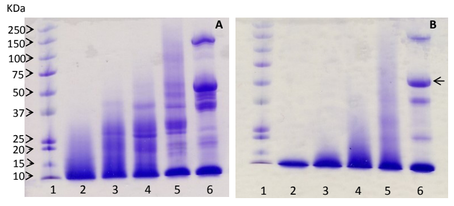


Figure 1. 10-15 % SDS-PAGE PhastGel of ricin extract following addition of varying concentrations of NaOH. (A) 30 minutes post addition (B) 24 hours post addition. Lane 1 – molecular weight marker, Lane 2 – 10 % NaOH, Lane 3 – 5 % NaOH, Lane 4 - 2.5 % NaOH, Lane 5 – 1% NaOH. Lane 6 – Untreated ricin extract. The ricin band at 66KDa is marked with an arrow in lane 6 on gel B.

Medium Scale Assessment

10% NaOH was taken forward asthe optimal concentration and assessed against a larger volume of ricin extract and with the NaOH added as a solid.

NaOH pellets were added to three replicates of 100 mL of crude ricin extract to give a final concentration of 10 % w/v NaOH. Following stirring samples were taken at time 0, which was considered when no visible pellets were present and then after 30 minutes, 1hour, 2 hours, 4 hours and 24 hours incubation. Samples were again analysed by HHA and SDS-PAGE. The temperature of the extract was also recorded at these time points.

The VSC scores (Table 2.) and SDS-PAGE (data not shown) results followed the same trend as the small scale assessment. The VSC results indicated that the rate of hydrolysis to be faster than in the small scale studies.

	Rep 1		Rep 2		Rep 3	
	HHA VSC Score	Temp ºC	HHA VSC Score	Temp ºC	HHA VSC Score	Temp ºC
Before treatment	10	16	10	16	10	16
t=0	8	32	8	32	8	33
t=30 min	3	25	3	25	3	25
t=1hour	2	22	2	22	2	22
t=2 hour	-ve	21	-ve	21	-ve	21
t=4 hour	-ve	20	-ve	20	-ve	20
t=24 hour	n/a	20	n/a	20	n/a	20

Table 2. Hand Held Assay VSC scores following the addition of NaOH pellets to ricin extract to a final concentration of 10 % w/v.n/a – assay not run

To assess this method on a larger scale, 4 litres of the simulant BSA at 40 mg/mL in 500mM NaCl pH4, was treated with NaOH pellets to a final concentration of 10 % w/v and the solution was stirred.As for the ricin extract no sign of intact protein was seen by SDS-PAGE after 1 hour; the reaction yielded a single band at the dye front after 24 hours.

Conclusions

- 10% w/v NaOH is the recommended concentration for the destruction of ricin
- A minimum incubation time of 4 hours is required for no structurally intact ricin to be detected.

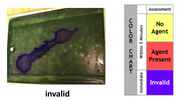
Enzyme-Based Disclosure Sprays for Nerve and Blister Chemical Warfare Agents

Scott Donahue, Jason Robosky, Beverly Rogers, Jonita Gidel, Jessica Milke, and Jeremy Walker, PhD

FLIR Systems, Inc., Pittsburgh, PA

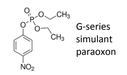
Agentase™ Disclosure Spray (ADS)

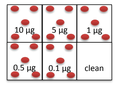
- Enzyme-based, aqueous formulation to detect chemical warfare agents on surfaces
  - Class-specific formulations for G- & V-series nerve agents or HD blister agent
  - Nerve agent formulation operates by inhibition of acetylcholinesterase
  - Colorimetric dyes indicate detection (yellow = clean, red = contaminated)
- Decontamination Applications
  - Contamination mapping
  - Enhanced decon efficiency
  - Post-decon assurance
- Reconnaissance Applications
  - Consequence management
  - Sensitive site exploitation
- Technology Applications
  - Standoff detection of surface contamination
  - Chemical agent sensing science
- Performance has been extensively validated via live agent testing
  - Edgewood Chemical Biological Command (ECBC) and Battelle
  - Various international test labs



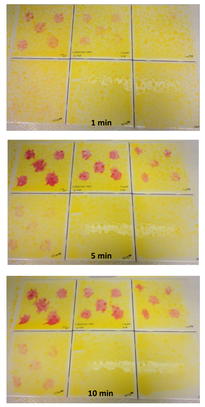
Nerve Agent Disclosure Spray

- Nerve Spray is yellow upon application
- In contaminated areas, the formulation enzyme is inhibited by nerve agents and the indicator dye turns red
- In clean areas, the dye remains yellow



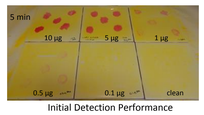


Agent	Detection limit Range	CIDAS Requirement
VX	**0.009 - 0.047 µg** (0.009 - 0.047 mg/m²)	0.4 {0.3} mg/m²
RVX	**0.004 - 0.009 µg** (0.004 - 0.009 mg/m²)	N/A
GD	**0.10 - 0.50 µg** (8-10 mg/m²)	16.7 {10.0} mg/m²
COI-1	1.4 - 7.7 ng (3.4 - 18.8 µg/m²)	N/A
COI-2	~0.77 ng (1.9 µg/m²)	N/A
COI-3	~7.7 ng (18.8 µg/m²)	N/A
COI-4	~1.4 ng (3.4 µg/m²)	N/A

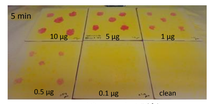


ADS Stability

- ADS R&D has focused heavily on formulation stability
- Shelf-life of packaged formulations is optimized through extensive studies involving accelerated aging at elevated temperatures
- Performance of reconstituted sprays remains stable for at least eight hours at temperatures as high as 40°C
- Standard quality control procedures include evaluation of ADS performance after incubation at 70°C for one week



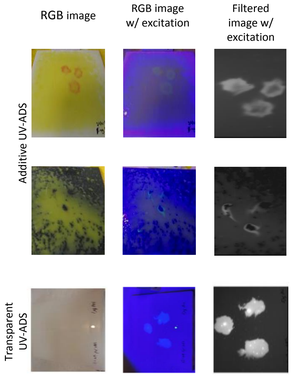
Initial Detection Performance



Aged One Week at 70°C

	RT	40°C	60°C	70°C	70-25 Cycle
Nerve ADS	est. >5 years	est. >3 years	>12 months	>3 months	>6 months
Blister ADS	est. >5 years	est. >3 years	8 months	3 months	6 months

UV Low Light Formulation



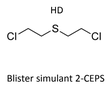
- Enhanced detection on dark surfaces
- Available in two options
  - An additive to standard Nerve ADS formulation
    - UV dye powder added to traditional ADS
    - Adds low light capability to existing product kits
    - No change in detection sensitivity
  - An optically transparent Nerve ADS formulation
    - Useful for FBI and SOCOM users
    - Looks like water on surface until illuminated
    - Leaves no visible residue upon drying
    - Commercial forensic kit compatible
    - Ideal for interior decontamination

Fielded ADS

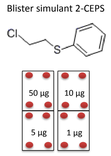
- ADS has been fielded in several kits with protection, identification, reconnaissance, and decontamination products
- Advanced Threat Detection (AT Boxes)
  - US Army 20th Support Command
  - 4 fielded
- Domestic Response Capability (DRC) Kits
  - National Guard WMD-CSTs
  - 57 fielded
- Dismounted Reconnaissance Sets Kits & Outfits (DR SKO)
  - Army, Air Force, Navy, Marines, and National Guard WMD-CSTs
  - 49 fielded

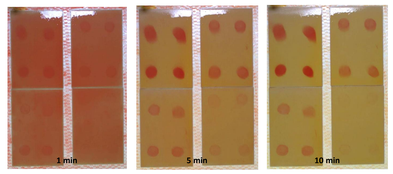
Blister Agent Disclosure Spray

- Blister Spray is red upon application
- In contaminated areas, the formulation enzyme is inhibited by blister agents and spray remains red
- In clean areas, the red dye becomes colorless, allowing visualization of the yellow background dye
- After signal development, the color scheme is similar to the Nerve Spray, with red detections on a yellow background



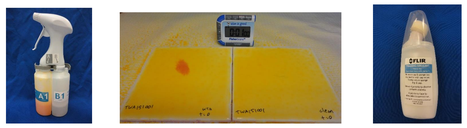
Detection limit Range	CIDAS Requirement
<10 µg (<15 mg/m²)	33 {15} mg/m²





User Training/Confidence

- Training ADS uses a benign simulant to mimic the detection response of Nerve ADS for end user instruction



- Confidence Check Cards for Nerve, Blister, and Training Sprays give the operator increased assurance that sprays are functional prior to application

Human Health Risks/Environmental Impact

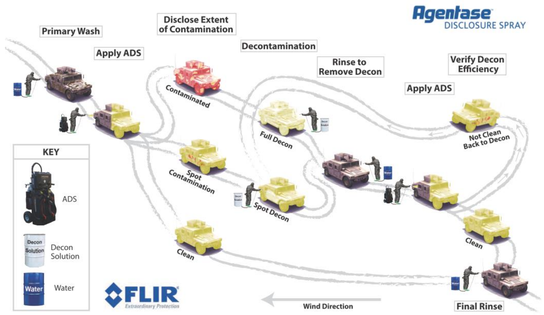
- Thorough User Health Risk and Environmental Impact Study performed by consulting firm
  - Hazard Index (HI) = $HQ_{ingestion} + HQ_{dermal} + HQ_{inhalation}$
  - All HI<1, indicates no potential for adverse health effects to occur
  - ADS is compliant with all applicable regulations (scale considerations)
  - ADS does not pose a hazard to users even without IPE during typical use or accidental release
  - ADS does not pose an ecological hazard to aquatic or terrestrial model organisms

Spray Formula	Direct HI	Indirect HI
Exposure to Liquid Residue During Typical Use / Accidental Release (Indirect via Soil)		
Training	**1E-02 / 3E-03**	**9E-07 / 2E-05**
Nerve	**7E-02 / 8E-03**	**8E-07 / 2E-05**
Blister	**6E-01 / 2E-01**	**7E-05 / 2E-03**
Exposure to Liquid Residue in Surface Water During Typical Use or Accidental Release (Direct Powders)		
Training	**2E-04**	**1E-07**
Nerve	**1E-03**	**3E-07**
Blister	**4E-03**	**8E-06**

CIDAS Program of Record

- Contamination Indicator Decontamination Assurance System (CIDAS)
  - Participating services include Army, Air Force, Marines, and SOCOM
- Managed by Joint Project Manager Protection (JPM P)
  - Fields all equipment in support of field decontamination (PPE, CPE, decontaminants, etc.)
- FLIR is prime contractor to provide indicator formulation (ADS)
  - Small Scale (0.5 L) Systems and Large Scale (2 gal) consumables
  - Nerve and Training indicator formulations and Confidence Check Cards (Blister formulation expected to transition in 2017)
- 10 year program
  - Engineering and Manufacturing Development Phase (2015-2017)
  - Low Rate Initial Production Phase (2017-2019)
  - Full Rate Production Phase (2019-2025)

Decon Triage with ADS



Form Factors

- ADS is available in several form factors to fit application needs
  - Screening sensitive equipment for contamination
  - Personnel screening
  - Sensitive site assessment
  - Decon assurance/verification
  - Equipment/vehicle screening
  - Consequence management
  - Mapping wide area contamination for decontamination

Specification	Handheld	Mid-scale	Large-scale
Capacity (liters)	0.5	7.57	53
Coverage (square meters)	5-7	75-100	550-700

Acknowledgements

- Defense Threat Reduction Agency
- Army Research Office
- Hazard Mitigation, Materiel, and Equipment Restoration Advanced Technology Demonstration (HaMMER ATD)
- Joint Project Manager for Protection
- Warfighters

Contact:
Scott Donahue
Project Manager, FLIR Detection
2240 William Pitt Way, Pittsburgh, PA 15238, USA
Email: scott.donahue@flir.com | www.flir.com

Lewisite and VX Degradation By-Product Method Development for Environmental Remediation by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS)

Stuart A. Willison[1], Terry M. O'Neill[2], Sandip Chattopadhyay[1], Carolyn Koester[3], Deon Anex[3], Romy Campisano[1], Matthew Magnuson[1]

[1]U.S. Environmental Protection Agency, [2]MRIGlobal, [3]Lawrence Livermore National Laboratory

Stuart Willison | Willison.stuart@epa.gov

www.epa.gov/research

Introduction

Analytical detection methods for conventional chemical warfare agents (CWAs) were mostly derived for military purposes and did not focus on the environmental aspect nor intended for use when dealing with contaminated civilian areas. If civilian areas are affected (e.g., Tokyo, Japan in 1995 and Syria in 2013), then it is necessary to ensure that analytical detection methods are appropriate for a particular analyte in the desired matrix. CWA degradation by-products can be extremely hazardous to humans, with similar toxicity values as the parent CWA, and environmentally persistent compared to parent agent. Therefore, it is important to have available analytical methods to detect the parent CWA and its degradation products in environmental matrices.

Impact

The presence of degradation products can assist decision-makers with identifying the extent of contamination, potentially high-concentrated areas of the parent and/or degradation products, and decontamination efficacy and/or remediation completeness necessary for civilian reoccupation.

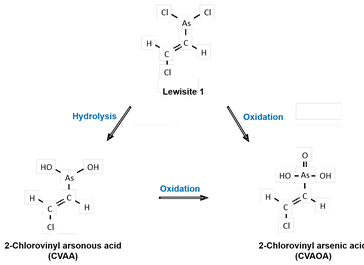


Figure 1. Lewisite 1 hydrolysis and oxidative pathways under environmental conditions.

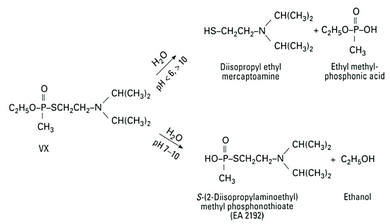
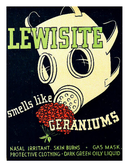


Figure 2. VX hydrolysis pathway under environmental conditions.

Identified Gaps and Method Application

Lewisite 1 is a reactive vesicant agent. Since arsenic is present in a variety of agricultural chemicals and in natural sources, assaying for elemental arsenic lacks specificity required for source material identification. Neither Lewisite or degradates (Figure 1) exist in the environment.



- Gas chromatographic (GC) analysis of Lewisite or CVAA presents difficulties, including GC inlet reactivity/corrosion, column deterioration, and clogged syringes.
- CVAA and CVAOA are environmentally persistent and unique, unambiguous indicators for Lewisite.
- The new method addresses sampling and analysis for CVAA and CVAOA in soil, wipe extracts, and water by LC-MS/MS.
- Method directly addresses need for Lewisite and degradate analysis procedures.

VX is considered to be one of the most toxic CWAs ever produced. Under certain pH conditions, VX will degrade to EA-2192 (Figure 2), which is environmentally persistent and possesses toxic properties similar to VX. Analysis methods following strict data quality objectives (DQOs) are needed for detecting EA-2192 in drinking water at environmentally low and health-based levels.

- U.S. EPA Method 538 analytical conditions and were adapted and performance metrics were applied for the determination of EA2192 in drinking water.
- Laboratories are familiar with EPA 500 series methods, thus, the capability to analyze EA2192 with the rigorous conditions of Method 538 will increase the analysis capacity following a VX release and subsequent remediation activities.

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U.S. Environmental Protection Agency
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Method and Results

Lewisite 1 hydrolyzes quickly in the environment to CVAA, then oxidizes more slowly to CVAOA. The developed method extracts Lewisite 1 and/or degradation products from the matrices of interest and converts them to CVAOA prior to analysis. Phenyl arsonous acid (PAA), which can be oxidized to phenyl arsonic acid (PAOA), was used as a surrogate to show that both extraction and oxidation processes were successfully implemented. The use of LC-MS/MS provides analytical specificity, by which degradation products of Lewisite 1 can be more easily measured (Figure 3) in the presence of interfering compounds.

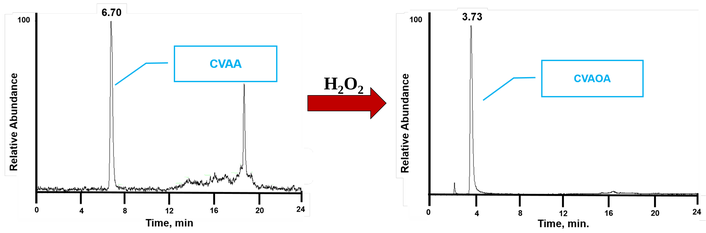


Figure 3. Chromatograms depicting CVAA analysis and oxidation to CVAOA by LC-MS/MS.

Stock and spike solutions consisted of Lewisite in water. The extraction, sample preparation, and analysis results are provided in Tables 1 and 2. Transformation of Lewisite 1 to CVAA and subsequent oxidation to CVAOA, by hydrogen peroxide (H_2O_2) was investigated and concluded that complete conversion was successful.

	Water	Wipes	Soil
Extraction	None	Shaker table for 30 min with 10 mM HCl	Shaker table for 30 min with 10.0 mL 50/50 (v/v) 10 mM HCl /methanol
Oxidation	1:1 Dilution with 30% H_2O_2	1:1 Dilution with 30% H_2O_2	1:1 Dilution with 30% H_2O_2

Table 1. Sample extraction (of CVAA) and oxidation (to CVAOA) procedures in tested matrices.

Method detection limits (MDLs) and recoveries for all tested matrices are reported in Table 2 and compared to risk-based health levels. A 14 day stability study was performed under refrigerated conditions (4 ° C ± 2 ° C) for Lewisite by-products. Statistical analysis suggests that Lewisite degradates were stable in water and on wipes, but significant differences were observed (< 2 days) for soil types.

Matrix	Spiked CVAA Concentration	Measured CVAOA Concentration (avg ± std)	Recovery (%)	MDL for CVAOA	ATL[a]
Water (µg/L)	0.20 e-3	0. 22 e -3 ± 0.01 e-3	110	0.04 e-3	0.03 e-3
Wipe (µg/wipe)	3.0	3.0 ± 0.1	101	0.4	-
Sand (µg/g)	0.20	0.17 ± 0.02	85	0.07	0.3
NB Soil (µg/g)	0.20	0.22 ± 0.01	112	0.03	0.3
VA Soil (µg/g)	0.40	0.17 ± 0.01	43	0.03	0.3
GA Soil (µg/g)	0.40	0.32 ± 0.02	80	0.05	0.3

Table 2. Analytical recoveries (n=7), MDLs, and Analytical Target Levels (ATLs) for Lewisite 1 method. [a]ATL values based on U.S. Army Public Health Command Chemical Agent Health-Based Standards and Guidelines Summary Table 2: Criteria for Water, Soil, Waste, 7/2011.

Method and Results

Water samples fortified with VX degradation product (EA-2192) were analyzed by LC-MS/MS. The compound was evaluated using EPA Method 538 conditions, including accuracy, precision, reproducibility, linearity, detection limit, and quantitation limit and results are presented in Table 3. The retention time for EA-2192 was 5.4 minutes. Target chemicals, Methamidophos and Methamidophos-d_6 (used as an internal standard), were used to assess method performance.

EPA Method 538 Performance Metrics Applied to EA-2192	Results
Calibration Curve Accuracy at Calibration 1 (0.050 µg/L)	96.4-105%
Calibration Curve Accuracy at Calibration 2-7 (< 5.00 µg/L)	92.4-107%
Laboratory Reagent Blank	ND
Method Precision at Calibration 4 (0.480 µg/L)	9.61%
Method Accuracy at Calibration 4 (0.480 µg/L)	21.8%
Method Detection Limit (µg/L)	0.013
Method Reporting Limit (µg/L)	0.125
Risk-Based Health Level* (µg/L)	0.021

*Table 3. U.S. EPA Method performance criteria applied to EA-2192. *Risk-Based Criteria to Support Validation of Detection Methods for Drinking Water and Air, EPA/600/R-08/021, 2008.*

A stability for EA-2192 was performed using water from four unique water utility companies, representing four different water types (Table 4). Storage stability of EA-2192 was evaluated for 28 days under refrigerated conditions (5 ° C ± 3 ° C). The average EA-2192 concentration in all four water types after the tested period was 99.2 % of the Time 0 results. Chlorinated water was also tested (Cl at ~1 mg/L, representing a typical Free Chlorine level in drinking water).

Water Type	Day 28 as a % of Day 0
In-house Deionized (DI) Water	86.7 %
Low TOC, chlorinated surface water	91.3 %
High TOC, chlorinated surface water	93.4 %
Low TOC, chlorinated surface water	99.7 %
High hardness, chlorinated surface water	112 %
DI Water + 1 mg/L free Cl, no preservatives	ND at Day 0

Table 4. Stability studies for EA-2192 in different water sources. TOC = Total Organic Carbon.

Conclusion

A novel LC-MS method was developed for CVAA and CVAOA in environmental matrices. The presence of CVAOA and/or CVAA is indicative of Lewisite 1 contamination, as there are no known natural sources for these compounds. The method was successfully tested with real-world samples suspected of containing Lewisite. In addition to the target analytes, the LC-MS method analyzed for thirteen additional As by-products, not possible by GC-MS or ICP-MS analysis techniques, and correctly identified the arsenic contamination.

U.S. EPA Method 538 performance metrics were successfully applied to EA-2192. Stability studies suggest that the toxic by-product is stable in drinking water sources. Other nonvolatile CWAs potentially can be tested using Method 538.

Both methods successfully fulfill an Agency gap by addressing lab capacity and capability for analyzing environmental samples containing toxic CWAs and their degradation by-products.

DISCLAIMER: **The U.S. Environmental Protection Agency through its Office of Research and Development (funded and managed) or (partially funded and collaborated in) the research described here under (contract number) or (assistance agreement number) to (contracting company name). It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.**

Chemical Hazard Mitigation Efficacy of Dahlgren Decontaminant

Bruce Campbell, Erin Lamb, Amy Andrews, Melany Corlew, Ellen Engelhart, and George Wrenn, Battelle HMRC
Kathryn Burns and Bryan Tienes, NSWCDD; Amit Kapoor and Randy Sakowitz, First Line Technology, LLC

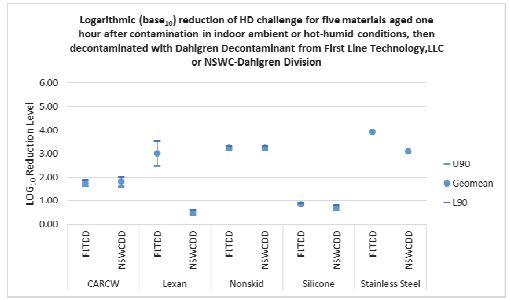
ABSTRACT

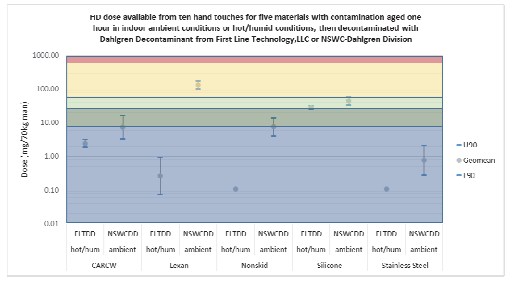
Chemical decontamination efficacy tests against persistent nerve agent (VX) and distilled mustard agent (HD) were performed at the Battelle Hazardous Materials Research Center (HMRC) to assess the hazard mitigation performance of Dahlgren Decontaminant prepared by First Line Technology, LLC (FLT) and by the Naval Surface Warfare Center Dahlgren Division (NSWCDD). Contact transfer and remaining contamination tests were performed on coupon samples of five materials: chemical agent resistant coating, water dispersible (CARCW), polycarbonate (Lexan), ship deck coating (Nonskid), silicone rubber, and stainless steel (17-4PH) that are typically representative of military vehicles and equipment.

Test procedures, calculations, and analyses were performed following U.S. Army Test Operations Procedure (TOP) 8-2-061A "Chemical Decontaminant Testing" [1], using detailed practices described in ECBC-TR-980 "The Chemical Contaminant and Decontaminant Test Methodology Source Document, Second Edition" [2]. Contact transfer results were compared to chemical exposure hazard levels published in USACHPPM 47-EM-5863-04 "Acute Toxicity Estimation and Operational Risk Management of Chemical Warfare Agent Exposures" [3] using hand surface area estimates from Yu, et.al., Burns. 2008 Dec;34(8):1183-9 [4] and Sheridan, et.al., J Burn Care Rehabil. 1995 Nov-Dec;16(6):605-6 [5].

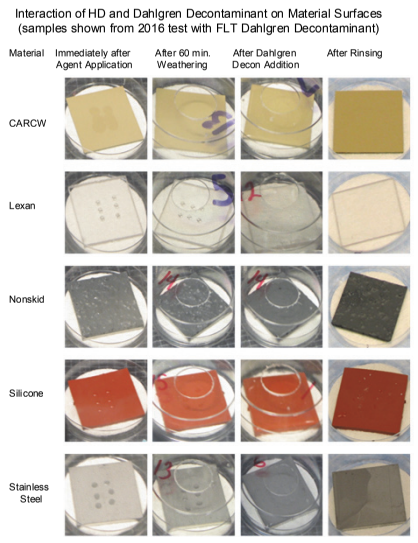
Dahlgren Decontaminant formulations from FLT and NSWCDD exhibited similar chemical hazard mitigation performance against each chemical warfare agent on each test material. In general, contamination levels were reduced by at least two orders of magnitude on coatings and hard materials. Differences in decontamination performance were observed on polycarbonate (Lexan) with HD and on Navy Nonskid with VX. Differences were caused by variations in the materials, themselves, not the decontaminant. Tests with the FLT formulation used SABIC Margard MR-10 polycarbonate and Navy Nonskid Compound-G (Type-I), while tests with the NSWCDD formulation used SABIC GE Lexan XL-10 and a sprayable Nonskid coating (Type-IV).

The chemical agent contact exposure hazard was reduced to negligible risk levels (i.e., no noticeable effect) on most materials for both chemical agents, even with repeated direct contact (i.e., ten hand touches). Reduction of contamination levels on silicone rubber was less than one order of magnitude for both HD and VX. Repeated direct contact with HD on silicone would be sufficient to produce noticeable but not disabling health effects. Incidental contact (i.e., one hand touch) with VX on silicone could produce disabling or incapacitating injuries, and repeated direct contact with VX on silicone or Compound G Nonskid could be sufficient to produce death, severe disabling, or incapacitating injuries without prompt medical stabilization and access to field or fixed site hospital facilities.





HD Risk Levels ([3] Table 5-4)	Below Negligible	Negligible (6-28mg)	Marginal (28-60mg)	Critical (60-600mg)	Catastrophic (above 600mg)



TECHNICAL APPROACH

The decontamination efficacy tests for FLT Dahlgren Decontaminant were conducted in 2016. Two trials were performed with each chemical agent at controlled hot/humid conditions (35±3°C, 70±10%RH). Tests for the NSWCDD formulation were conducted in 2012. Three trials were performed with each chemical agent at indoor ambient conditions. Each trial included one chemical agent, Dahlgren Decontaminant as one hazard mitigation option, and multiple coupons of each designated test material.

Chemical agent was applied to each coupon using a 100-µL syringe to administer 2-µL droplets of HD or VX for a nominal contamination density of 10 g/m^2. Three dose confirmation samples were prepared during each trial to verify operation of the agent delivery tool during application of agent to test coupons.

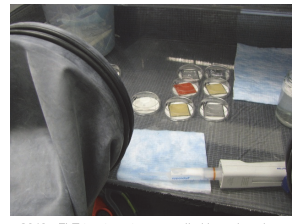
Dahlgren Decontaminant was prepared in a 200 mL batch for each trial following manufacturer's specifications. Decontaminant was applied as a single dose to each coupon using a positive displacement pipette to cover the contaminated coupon surface and achieve an application density of 1250 mL/m^2. Each batch of Dahlgren Decontaminant was prepared immediately prior to application and was used on all coupons in the trial within 30 minutes. Residence time for the decontaminant on each coupon was 30 minutes.

At the end of the decontamination period, each coupon was rinsed thoroughly with distilled water (2x2 mL/cm^2 front and 2x1 mL/cm^2 back) following laboratory treatment protocol, and allowed to dry in air for 30 minutes at indoor ambient conditions. Researchers recorded the presence or absence of any remaining water droplets on coupon surfaces, and then wicked the last remaining water beads off coupon surfaces using pieces of plastic-backed blotting paper.

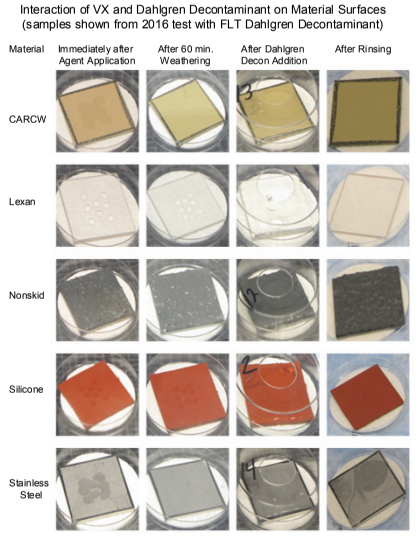
Coupons were transferred to a heated plate set to 30±3°C for the contact transfer testing. Latex dental dam (LDD) samplers were applied to each coupon to obtain contact transfer samples. The LDD was covered with an aluminum foil barrier between the LDD and a contact sample weight (pre-heated to 30°C). The weight produced 1 psi contact force and was kept in place for a period of 15 minutes. For Navy Nonskid coupons, a soft paper towel material was also placed in between the weight and the foil to avoid only applying pressure to the higher points of this uneven surface.

At the completion of the contact time, the weight was removed from each sample. The LDD and foil were placed in a jar containing 20 mL hexane for extraction. The coupon was placed in a separate extraction jar containing 20 mL hexane. Extraction times of one hour, minimum, were used for all samples. At the end of the extraction period, 1 mL aliquots of each sample were transferred into sample vials for quantitative analysis using either gas chromatographs equipped with flame photometric or flame ionization detectors, or a liquid chromatograph coupled to a triple quadrupole mass spectrometer.

2012 - NSWCDD treatments are applied in a laboratory hood at indoor ambient conditions



2016 - FLT treatments are applied in a glove box at hot/humid conditions (35±3°C/70±10%RH)



RESULTS

The contact transfer mass per unit area for a single touch (T1[M]) obtained from each contact (LDD/foil) sample and agent residue extraction mass per unit area (RE[M]) obtained from each coupon sample were determined using quantitative measurements of agent concentration in analytical samples.

T1[M] = T1[E] x EV ÷ CA x SC
RE[M] = RE[E] × EV ÷ CA x SC
Where: T1[M] or RE[M] = Mass per unit area (mg/m^2)
T1[E] or RE[E] = Agent concentration in analysis sample (µg/mL)
EV = Extraction solvent volume (20 mL)
CA = Coupon area (14.5 cm^2)
SC = Scale constant (10,000cm^2/m^2 ÷ 1,000µg/mg)

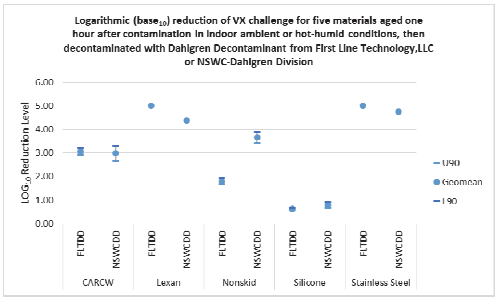
Decontamination efficacy for each material was determined by evaluating the overall reduction of contamination (RSC[RA]) from the surface. RSC[RA] is the ratio between initial contamination challenge per unit area (DC[M]) and remaining agent level per unit area (RA[M]), expressed as an order of magnitude (base[10]) logarithmic value. DC[M] was determined from quantitative measurements of agent concentration in dose confirmation samples. RA[M] was calculated by combining the amounts obtained from contact transfer and agent residue extraction samples.

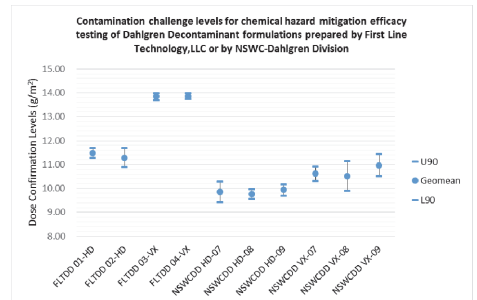
RA[M] = T1[M] + RE[M]
DC[M] = DC[E] x DCEV ÷ DCCA x DCSC
Where: DC[M] = Dose mass per unit area (g/m^2)
DC[E] = Agent concentration in dose confirmation sample (µg/mL)
DCEV = Dose extraction solvent volume (50 mL or 80 mL)
DCCA = Dose coupon area (10.00 cm^2 or 14.52 cm^2)
DCSC = Dose scale constant (10,000cm^2/m^2 ÷ 1,000,000µg/g)

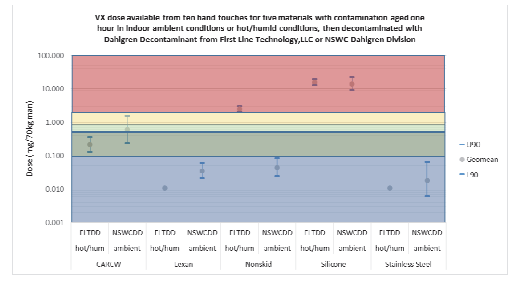
Chemical agent contact exposure health hazard effects were estimated as the cumulative dose delivered to a 70 kg man if he touches a decontaminated surface repeatedly (i.e., ten times) with his whole hand, each hand touch to a fresh location on the surface, and receiving the full available contact mass (T1[M]) in each touch.

Surface area of the human hand (palm region) represents 0.85% of body surface area [4],[5].

Repeated contact is conservatively defined as ten separate hand touches, each touch to a fresh location on the material surface. Each hand touch lasts for up to 15 minutes duration. Ten touches represents cumulative contact time of 2-1/2 hours.







VX Risk Levels ([3] Table 5-4)	Below Negligible	Negligible (0.08-0.55mg)	Marginal (0.55-0.80mg)	Critical (0.80-2.0mg)	Catastrophic (above 2.0mg)