# Report on The 2015 U.S. EPA International Decontamination Research and Development Conference

# Appendix C

## Presentation Slides

# Improved Filter Holder and Extraction Protocol for Forensic Vacuum Collections

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MRIGlobal
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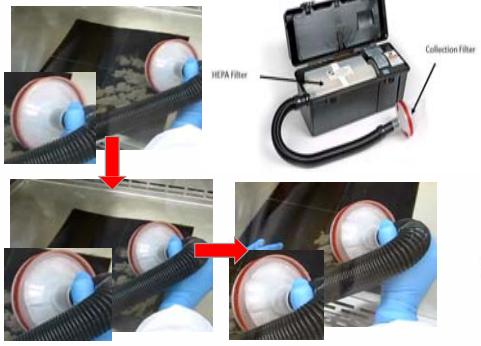
## Introduction

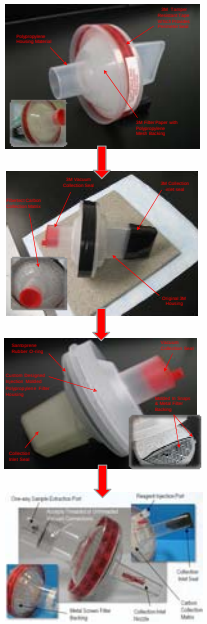
In response to a need for improved collection for bioforensic evidence, MRIGlobal has developed the Bioforensic Collection Filter (BCF) using Fibertect fabric to address collection performance deficiencies in the 3M Trace Evidence Filter. It has been estimated that as many as 20% of 3M Trace Evidence filters fail during collection. The most common failure is attributed to loss of matrix integrity and/or failure of filter holder to properly retain filter, both of which can lead to loss of evidence.

## Background

In general, vacuum filtration is a portable and effective method for easily sampling biological particulates from large diverse surfaces including wood, metal, and carpet. Current vacuum collection systems, such as the 3M Trace Evidence Collection System shown below, employ a vacuum fitted with a collection filter. The replaceable collection filter is hermetically sealed and installed on the vacuum nozzle with a friction fitting. The vacuum is also equipped with a HEPA filter to prevent any collected particles from being exhausted through the blower stage during collection. Some of the issues encountered with this system include loss of filter integrity (pictured below) during collection and difficulty recovering targets during sample extraction due to the hydrophobic nature of the filter media. Additionally, the vacuum motor can overheat during collection and shut off, which often leads end-users to forgo the system completely for more reliable COTS vacuums.




## Engineering Design Evolution



3M TRACE EVIDENCE FILTER

The 3M Trace Evidence Filter was found to have a 20% failure rate during vacuum collections. These failures can be attributed to the polypropylene filter backing buckling under high vacuum (such as when filter clogs) as well as the 3M filter matrix rupturing during vacuum collections . Furthermore the physical design of the 3M filter holder makes the post-collection extraction method cumbersome and unsafe because 1) the perimeter seal must be manually cut and 2) the filter holder must be opened, which exposes the operator to dangerous aerosols.

YEAR 1 DESIGN

During initial evaluation and development of the BCF as a forensic collector, MRIGlobal used the 3M Trace Evidence Filter housing as there was no other commercially available housing that would accommodate the diameter of the filter. While adequate for holding the matrix under test, it was not optimized for the thicker Fibertect fabric filter and failed to completely constrain the matrix. Leak testing performed by MRIGlobal engineers showed that the current 3M filter collection inlet seal and vacuum collection seal were not suited for *in situ* extraction methods.

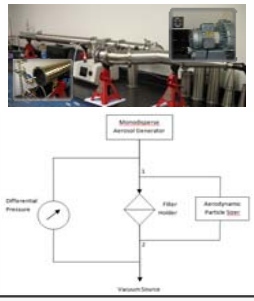
YEAR 2 DESIGN

In year 2, MRIGlobal engineers addressed the filter housing failure issue by designing a custom injection molded polypropylene filter housing. This design utilized an aluminum mesh filter backing with 70% open area to prevent the filter from buckling under the stress of vacuum collection while minimally changing the pressure drop across the filter cross section. A custom collection inlet seal was also designed and the diameter of the vacuum collection port was reduced and molded in snaps were used to hold pressure against a polymeric perimeter seal to prevent leaks during *in situ* extractions.

YEAR 3 DESIGN

Year 3 was aimed at designing a more effective seal for the Fibertect Filter Holder during *in situ* extractions while also improving the ease of use during the extraction process. The improved design features a collection inlet seal that is more effective than the previous year's and is easier to remove and replace (based on feedback from end-users). The perimeter and vacuum collection port seals were also modified to include threaded connections that seal the filter holder with o-rings. One-way reagent injection and sample extraction ports were added to allow the user to easily inject or remove liquid from the filter holder via a syringe. Finally, the filter holder was constructed from clear ABS which allows the user to see into the housing and provides ruggedness.

## Engineering Testing and Evaluation




MRIGlobal performed collection efficiency comparison testing between the 3M Trace Evidence Filter Matrix and the BCF Matrix. This testing was performed with the setup shown to the left with a mono-disperse aerosol of 1, 4.5, and 10µm polystyrene latex microspheres. The microspheres were drawn into the system by vacuum and captured by the matrix candidate installed inline. Particle concentration measurements were made at locations 1 and 2 with a TSI 3321 APS. Particle capture efficiency was calculated as the ratio of particles at position 2 to position 1. Differential pressure measurements across the matrix candidate were made concurrently with particle concentration measurements.

## Extraction Protocols

3M Extraction Protocol

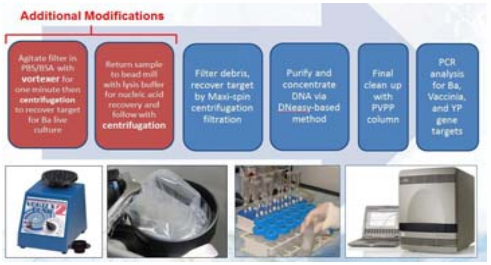
Use of the 3M Trace Evidence Filter is complicated by the post-collection processing method, which requires opening the housing and removing the filter. This approach can increase the chance for sample loss and contamination of biosafety cabinets used for sample processing. These issues increase the time and expense of sample processing and jeopardize the integrity of the forensic sample.

BCF Extraction Protocol

Extraction from the BCF involves an *in situ* extraction method that requires no opening or removal of the filter and therefore preserves the integrity of the collected evidence and prevents contamination of the collected sample while promoting greater recovery and limiting losses during processing.

## Biological Testing and Evaluation

MRIGlobal tested the BCF in operationally relevant test scenarios with high replicates to provide statistical confidence in performance limits. These studies addressed extraction validation criteria to include target range, limit of detection and the quantity of target needed for subsequent live culture and PCR analysis.




Live culture study was performed with *Bacillus anthracis*. The filter units were seeded at 1E8 - 1E2 CFU/filter with various background materials from .5 - 2.0 g/filter. Testing showed that a 1 g/filter background load and 1E3 CFU/filter spiking concentration provided the best live culture results

## Engineering Results

During al tests, sample flow rate was maintained at 1100 liters/minute, which corresponds to the measured collection flow rate of the 3M Trace Evidence Vacuum. This flow rate resulted in a face velocity at the filter of 2.6 meters/second. Average pressure drop across the filter at the sample flow rate for each candidate is shown below in Table 1. Pressure drops did not vary significantly during the course of the evaluation and were under the 13.7kPa limit for the 3M Trace Evidence Vacuum as specified by 3M.

**Table 1. Average Pressure Drop Data for Matrix Candidates**

| Filter type | Average pressure drop (Pa) |
|---|---|
| 3M Trace Evidence Collection Filter | 1500 |
| Fibertect Decontamination Fabric | 2000 |
| High Volume Chemical Sampler | 3200 |

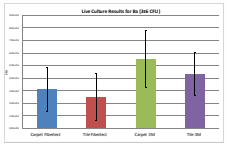
Particle capture efficiency data for the matrix candidates are shown below in Table 2. Particle capture efficiency is a ratio of the number of particles retained by the filter to the number of particles available for capture by the filter and provides an indication of how well filters retain particles that are picked up by the vacuum. These results show that the Fibertect® matrix exhibits greater capture efficiency over the target particle range when compared with the 3M filter.

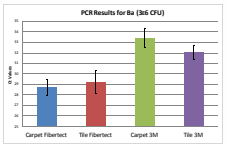
**Table 2. Average Particle Capture Efficiency Data for Matrix Candidates**

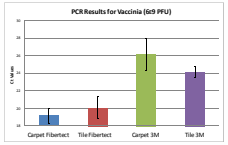
| Filter Type | Particle diameter | | |
|---|---|---|---|
| | 1 µm | 4.5µm | 10 µm |
| 3M Trace Evidence Collection Filter | 73.3% | 69.2% | 70.6% |
| Fibertect Decontamination Fabric | 99.7% | 99.9% | 94.1% |
| High Volume Chemical Sampler | 99.7% | not tested | not tested |

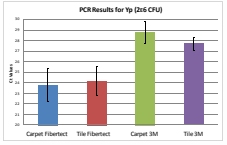
## Live Culture and PCR Results

MRIGlobal executed molecular and microbiological comparison testing between the 3M Filter Matrix and the Fibertect Forensic Filter Matrix. This testing aimed to prove that the Fibertect Filter Matrix performed as good as or better than the 3M Filter Matrix in relevant collection situations. The results of this testing are shown in the charts below.




Year 3 consisted of range finding studies from 1e5 to 1e2 which yielded the limit of reliable detection through PCR for *Bacillus anthracis* (5e3 seeding level), Vaccinia virus (1e3 seeding level), *Yersinia pestis* (1e3 seeding level). To prove statistical robustness of the extraction method 62 samples were processed for the range finding study, precision studies, consisted of two operators and 48 samples.

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# Evaluation of chlorine dioxide and ozone formulations for soil sanitation

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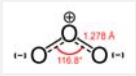
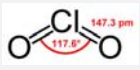
## Introduction

Due to the phase-out of methyl bromide, alternative methods are needed for nursery soil sanitation. This study was conducted to explore various oxidant biocide formulations for deactivating pathogens in nursery soils. Several biocides were tested in a greenhouse soil column study: ozonated water, liquid chlorine dioxide, chlorine dioxide granules (fumigant), steam treatment (autoclave) and untreated soil (control). We evaluated the effects of soil type (commercial top soil or potting soil) and the effect of repeated biocide applications (2, 4 or 6 applications) for ozonated water and liquid chlorine dioxide.



Ozone



Chlorine Dioxide

## Methods

Acrylonitrile Butadiene Styrene soil columns measuring 30.5 cm long with a 10.2 cm inside diameter were filled with commercial top soil or commercial potting soil (Fig. 1a). The soil surface was 4 cm from the top of the tube. For the potting soil and top soil, 700mL and 500mL, respectively, of liquid biocide were added to soil columns in 2, 4 or 6 weekly applications (Fig. 1b).


Fig. 1a: Soil columns in greenhouse


Fig. 1b: Liquid chlorine dioxide added to soil tube

We evaluated the effect of a single application of chlorine dioxide ($ClO_2$) granules to each soil type. $ClO_2$ gas was generated in the soil matrix by mixing two granular reagents (120 g per 2,058 $cm^2$ of soil; ICA TriNova Z-series) with moist soil. (Fig. 2). After granule application, tubes were covered with waxed paper for 5 days to trap the $ClO_2$ gas.

We also evaluated untreated control samples and soil samples that were autoclaved 3 times on days 0, 21 and 28 of the experiment (positive control).

All tubes were covered with a Sani-Cloth during the experiment to prevent microbial contamination. The cloth was only removed during biocide applications or for tube measurements.


Fig. 2a: Chlorine dioxide fumigant. The granules in the tubes are mixed and added to soil.


Fig. 2b: After granule application, white and tan granules mixed on soil surface

## Methods

Soil respiration ($CO_2$ concentration [efflux]) was measured 0, 23, 58, 79 and 93 days after the first biocide application using a LICOR 6400XT soil chamber head. We hypothesized that a reduction in the native microbial population in soils, due to the biocide treatment, would reduce soil respiration rates in the treated samples.

As an additional measure of antimicrobial efficacy, steel washers were inoculated with *Bacillus subtilis* spores (Fig. 3). *Bacillus* spores were selected because they may be a good model for disinfectant-resistant pathogens. Washers were inserted 10 cm into each soil column before the liquid biocide treatments, exposed to the biocides for 30 minutes, and then retrieved for culturing to determine viable spore counts. For the $ClO_2$ granules, washers were inserted 5 days after the granules were applied and removed after 30 min.

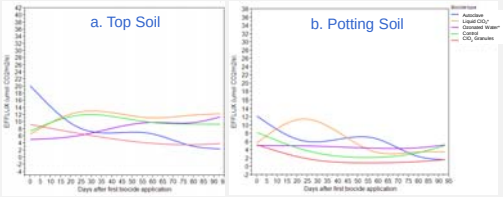
Mean efflux measurements were graphed using statistical smoother lines to join the points. For *B. subtilis* testing, $log_{10}$ spore reduction was calculated as: $log_{10}$ control CFU per washer counts – $log_{10}$ treatment CFU per washer counts.


Fig. 3: Steel washers inoculated with *Bacillus subtilis* spores

## Results

- A single application of chlorine dioxide granules resulted in a soil respiration rate equivalent to the autoclave treatment for the potting soil at 93 days after biocide application (Fig. 4b).
- For the top soil, the autoclave treatment had a slightly lower soil respiration rate than the chlorine dioxide granules after 93 days (Fig. 4a).

Fig. 4: Soil respiration means (µmol $CO_2/m^2/s$) over time

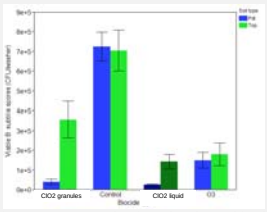


*Data shown are for samples that received 6 applications of the liquid biocides

- The chlorine dioxide liquid biocide had the lowest viable *B. subtilis* spore count (viable CFU/washer), which resulted in the highest efficacy rating among the three treatments that were tested with the inoculated spore samples (Fig. 5).
- Chlorine dioxide applied as a liquid, or as the granules, had an average $log_{10}$ *B. subtilis* spore reduction of 0.69 and 0.30, respectively, for an exposure time of 30 minutes, at 10 cm deep in top soil (Table 1).

## Results

Fig. 5: Viable *B. subtilis* spore counts after biocide treatment*



*Note: graph shows average counts, and not the results of the factorial modeling of the data

Table 1: *B. subtilis* efficacy results

| Biocide type | $Log_{10}$ reduction Potting soil | $Log_{10}$ reduction Top soil |
|---|---|---|
| ClO2 granules | 1.25 | 0.30 |
| Control | 0.00 | 0.00 |
| ClO2 liquid | 1.44 | 0.69 |
| $O_3$ | 0.68 | 0.59 |

## Discussion

The soil sanitation results differed between the soil respiration and the spore efficacy tests. Based on soil respiration results, the $ClO_2$ granules (fumigant) were equivalent to the autoclave for the potting soil, and almost equivalent for the top soil, at 93 days post-application. The autoclave was considered to be highly effective at reducing microbial populations. The liquid biocides showed very poor results, i.e., liquid biocides had high soil respiration rates compared to the control treatment at 93 days.

In contrast, the *B. subtilis* spore efficacy results showed that the liquid biocides had low viable spore counts compared to the control viable spore counts. The contrasting results between the soil respiration and spore efficacy results suggested that the method for measuring soil microflora is important. Soil respiration is a function of microbial respiration, chemical reactions in organic matter due to heat and chemicals, and amount of porosity or air space in the soil. Chemical reactions created by the liquid biocides may have generated carbon dioxide from the organic matter in the soil, which in turn may have confounded the interpretation of soil respiration rates in this study.

This study didn't analyze spore samples that remained in the soils over multiple biocide applications, so the cumulative effect of multiple applications could not be reported for the liquid biocides. In addition, higher spore efficacy may have been seen with the $ClO_2$ granules if the washers were inserted immediately after granule application. Washer application had to be delayed 5 days to prevent the ClO2 from immediately escaping from the tubes.

Future studies should include a method to sample the soil microbial population directly, to avoid any confusion with $CO_2$ generation by physical or chemical processes. The use of DNA barcoding or Petri dish plating with non-selective media could be used in future studies to measure microbial populations.

# Test method development for hot, humid air decontamination of materials contaminated with clean or dirty spores including *Bacillus anthracis*

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## OBJECTIVE

Methods to develop practical and statistical confidence in data sets for hot air decontamination were developed in order to assess technology using the synergistic action of heat, humidity and time as a biological decontaminant(s) for sensitive equipment without degradation of the functionality of that equipment. Evaluate the limits of the decontamination technology.

## NEED

There are no and/or limited sporicidal decontaminants that can be used on aircraft interior and/or sensitive equipment.

## MATERIALS AND METHODS

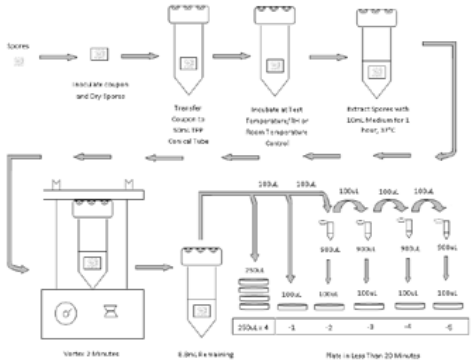

Figure 1. Step-by-step diagram of the hot, humid air decontamination method.

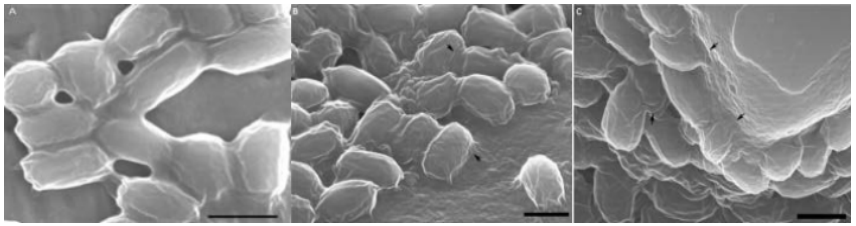

Figure 4. SEM micrographs of *B. anthracis* ΔSterne clean spores (A), spores mixed with humic acid (B), or spores mixed with kaolin (C). The interaction of the spore exosporium and the debris is highlighted by the black arrow. Size bars are 1.0 µm.

## RESULTS

| | | Relative humidity (RH) and time of decontamination | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 90% RH | 90% RH | 90% RH | 75% RH | 75% RH | 75% RH | 60% RH | 60% RH | 60% RH |
| Substrate | Temp (°C) | 1 day | 4 days | 7 days | 1 day | 4 days | 7 days | 1 day | 4 days | 7 days |
| Wiring insulation | 77 | 0±0 | NA | 0±0 | NA | 0±0 | NA | 5.7±2.9 | NA | 0±0 |
| Wiring insulation | 68 | NA | 0±0 | NA | 0.3±0.2 | **0±0** | 0±0 | NA | 0±0 | NA |
| Wiring insulation | 60 | 5.7±3.0 | NA | 0.2±0.2 | NA | 0±0 | NA | 7.2±0.2 | NA | 4.5±2.4 |
| APC | 77 | 0±0 | NA | 0±0 | NA | 0±0 | NA | 6.0±1.6 | NA | 0±0 |
| APC | 68 | NA | 0±0 | NA | 3.1±1.3 | **0.3±0.2** | 0±0 | NA | 3.6±1.8 | NA |
| APC | 60 | 6.5±0.6 | NA | 0±0 | NA | 3.9±0.7 | NA | 7.2±0.3 | NA | 5.3±0.3 |
| Anti-skid | 77 | 0±0 | NA | 0±0 | NA | 0±0 | NA | 5.6±1.4 | NA | 0±0 |
| Anti-skid | 68 | NA | 0±0 | NA | 3.9±1.5 | **0.8±0.4** | 0.1±0.2 | NA | 2.8±1.6 | NA |
| Anti-skid | 60 | 4.6±0.7 | NA | 0.8±0.6 | NA | 3.3±1.8 | NA | 6.9±0.3 | NA | 5.1±0.7 |
| Plastic | 77 | 0±0 | NA | 0±0 | NA | 0±0 | NA | 6.0±2.6 | NA | 0±0 |
| Plastic | 68 | NA | 0±0 | NA | 6.2±2.7 | **5.9±1.9** | 0±0 | NA | 0.3±0.2 | NA |
| Plastic | 60 | 7.1±0.3 | NA | 4.5±2.8 | NA | 5.0±1.7 | NA | 7.2±0.4 | NA | 6.4±0.3 |
| Nylon | 77 | 0±0 | NA | 0±0 | NA | 3.0±0.3 | NA | 7.3±0.3 | NA | 6.3±0.5 |
| Nylon | 68 | NA | 4.2±0.4 | NA | 7.1±0.1 | 7.0±0.2 | 6.6±0.2 | NA | 7.0±0.1 | NA |
| Nylon | 60 | 7.1±0.4 | NA | 6.9±0.4 | NA | 6.4±0.1 | NA | 7.3±0.2 | NA | 6.9±0.2 |
| Solution controls | 77 | 0±0 | NA | 0±0 | NA | 0±0 | NA | 0±0 | NA | 0±0 |
| Solution controls | 68 | NA | 0±0 | NA | 0±0 | **0±0** | 0±0 | NA | 0±0 | NA |
| Solution controls | 60 | 7.2±0.3 | NA | 2.7±1.8 | NA | 0±0 | NA | 7.0±0.2 | NA | 1.7±1.3 |

Table 1. Spore inactivation of clean *B. anthracis* ΔSterne spores.

| | | Relative humidity (RH) and time of decontamination | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 90% RH | 90% RH | 90% RH | 80% RH | 80% RH | 80% RH | 70% RH | 70% RH | 70% RH |
| Substrate | Temp (°C) | 1 day | 2 days | 3 days | 1 day | 2 days | 3 days | 1 day | 2 days | 3 days |
| Wiring insulation | 75 | 2.8±1.4 | NA | 0±0 | NA | 2.9±1.3 | NA | 7.0±0.1 | NA | 1.6±1.1 |
| Wiring insulation | 65 | NA | 7.0±0.1 | NA | 7.2±0.1 | **7.2±0.1** | 7.1±0.1 | NA | 7.2±0.0 | NA |
| Wiring insulation | 55 | 7.2±0.1 | NA | 7.2±0.1 | NA | 7.3±0.0 | NA | 7.1±0.1 | NA | 7.2±0.0 |
| APC | 75 | 5.8±0.3 | NA | 0±0 | NA | 4.7±1.0 | NA | 7.1±0.1 | NA | 4.0±0.7 |
| APC | 65 | NA | 7.2±0.2 | NA | 7.1±0.0 | **7.1±0.1** | 6.9±0.1 | NA | 7.2±0.1 | NA |
| APC | 55 | 7.2±0.0 | NA | 7.2±0.1 | NA | 7.3±0.1 | NA | 7.1±0.1 | NA | 7.2±0.0 |
| Anti-skid | 75 | 4.7±0.5 | NA | 0±0 | NA | 2.8±1.5 | NA | 6.6±0.3 | NA | 2.6±0.8 |
| Anti-skid | 65 | NA | 7.2±0.2 | NA | 7.1±0.0 | **7.0±0.2** | 6.9±0.1 | NA | 7.2±0.1 | NA |
| Anti-skid | 55 | 7.2±0.0 | NA | 7.2±0.1 | NA | 7.2±0.2 | NA | 7.1±0.1 | NA | 7.1±0.0 |
| Plastic | 75 | 6.0±0.3 | NA | 0±0 | NA | 4.2±0.8 | NA | 7.0±0.1 | NA | 3.6±0.9 |
| Plastic | 65 | NA | 7.2±0.2 | NA | 7.2±0.1 | **7.1±0.1** | 7.0±0.1 | NA | 7.2±0.1 | NA |
| Plastic | 55 | 7.2±0.2 | NA | 7.2±0.1 | NA | 7.3±0.0 | NA | 7.1±0.1 | NA | 7.2±0.0 |
| Nylon | 75 | 5.0±0.2 | NA | 0±0 | NA | 5.9±0.2 | NA | 7.0±0.1 | NA | 6.6±0.1 |
| Nylon | 65 | NA | 7.1±0.1 | NA | 7.1±0.2 | 7.0±0.2 | 6.9±0.1 | NA | 7.1±0.1 | NA |
| Nylon | 55 | 7.1±0.1 | NA | 7.2±0.1 | NA | 7.1±0.1 | NA | 7.1±0.1 | NA | 7.1±0.1 |
| Solution controls | 75 | 2.7±0.8 | NA | 0±0 | NA | 0±0 | NA | 1.6±1.6 | NA | 0±0 |
| Solution controls | 65 | NA | 6.7±0.4 | NA | 7.1±0.1 | **7.0±0.1** | 6.2±0.4 | NA | 6.7±0.3 | |
| Solution controls | 55 | 7.2±0.1 | NA | 7.2±0.1 | NA | 7.3±0.0 | NA | 7.1±0.1 | NA | |

Table 2. Spore inactivation of *B. anthracis* ΔSterne spores mixed with kaolin.

| | | Relative humidity (RH) and time of decontamination | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 90% RH | 90% RH | 90% RH | 80% RH | 80% RH | 80% RH | 70% RH | 70% RH | 70% RH |
| Substrate | Temp (°C) | 1 day | 2 days | 3 days | 1 day | 2 days | 3 days | 1 day | 2 days | 3 days |
| Wiring insulation | 75 | 2.8±1.4 | NA | 0±0 | NA | 2.9±1.3 | NA | 7.0±0.1 | NA | 1.6±1.1 |
| Wiring insulation | 65 | NA | 7.0±0.1 | NA | 7.2±0.1 | **7.2±0.1** | 7.1±0.1 | NA | 7.2±0.0 | NA |
| Wiring insulation | 55 | 7.2±0.1 | NA | 7.2±0.1 | NA | 7.3±0.0 | NA | 7.1±0.1 | NA | 7.2±0.0 |
| APC | 75 | 5.8±0.3 | NA | 0±0 | NA | 4.7±1.0 | NA | 7.1±0.1 | NA | 4.0±0.7 |
| APC | 65 | NA | 7.2±0.2 | NA | 7.1±0.0 | **7.1±0.1** | 6.9±0.1 | NA | 7.2±0.1 | NA |
| APC | 55 | 7.2±0.0 | NA | 7.2±0.1 | NA | 7.3±0.1 | NA | 7.1±0.1 | NA | 7.2±0.0 |
| Anti-skid | 75 | 4.7±0.5 | NA | 0±0 | NA | 2.8±1.5 | NA | 6.6±0.3 | NA | 2.6±0.8 |
| Anti-skid | 65 | NA | 7.2±0.2 | NA | 7.1±0.0 | **7.0±0.2** | 6.9±0.1 | NA | 7.2±0.1 | NA |
| Anti-skid | 55 | 7.2±0.0 | NA | 7.2±0.1 | NA | 7.2±0.2 | NA | 7.1±0.1 | NA | 7.1±0.0 |
| Plastic | 75 | 6.0±0.3 | NA | 0±0 | NA | 4.2±0.8 | NA | 7.0±0.1 | NA | 3.6±0.9 |
| Plastic | 65 | NA | 7.2±0.2 | NA | 7.2±0.1 | **7.1±0.1** | 7.0±0.1 | NA | 7.2±0.1 | NA |
| Plastic | 55 | 7.2±0.2 | NA | 7.2±0.1 | NA | 7.3±0.0 | NA | 7.1±0.1 | NA | 7.2±0.0 |
| Nylon | 75 | 5.0±0.2 | NA | 0±0 | NA | 5.9±0.2 | NA | 7.0±0.1 | NA | 6.6±0.1 |
| Nylon | 65 | NA | 7.1±0.1 | NA | 7.1±0.2 | 7.0±0.2 | 6.9±0.1 | NA | 7.1±0.1 | NA |
| Nylon | 55 | 7.1±0.1 | NA | 7.2±0.1 | NA | 7.1±0.1 | NA | 7.1±0.1 | NA | 7.1±0.1 |
| Solution controls | 75 | 2.7±0.8 | NA | 0±0 | NA | 0±0 | NA | 1.6±1.6 | NA | 0±0 |
| Solution controls | 65 | NA | 6.7±0.4 | NA | 7.1±0.1 | **7.0±0.1** | 6.2±0.4 | NA | 6.7±0.3 | |
| Solution controls | 55 | 7.2±0.1 | NA | 7.2±0.1 | NA | 7.3±0.0 | NA | 7.1±0.1 | NA | |

Table 3. Spore inactivation of *B. anthracis* ΔSterne spores mixed with humic acid + spent sporulation medium

### TIER II

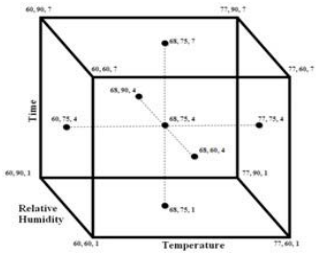

Figure 2. Response Surface Methodology (RSM) experimental design for three test factors (°C, % relative humidity, time in days). The center point is 68°C, 75% RH, 4 days.

### TIER III

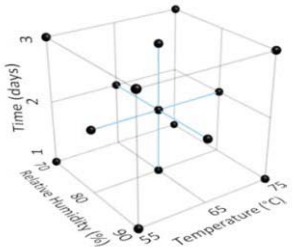

Figure 3. Response Surface Methodology (RSM) experimental design for three test factors (°C, % relative humidity, time in days). The center point is 65°C, 80% RH, 2 days.

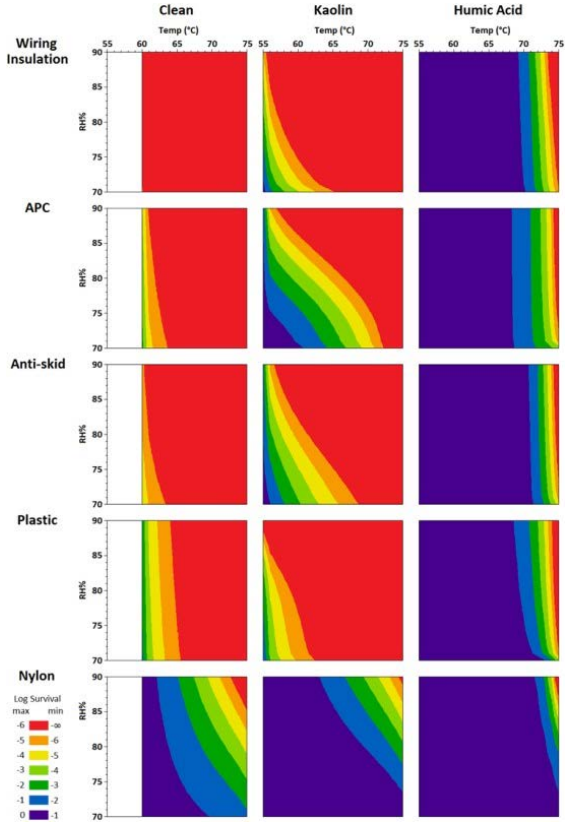

Figure 5. Models with a 90% statistical probability of a 6-log spore survival (purple) for *B. anthracis* ΔSterne spores (clean, kaolin, humic acid + spent sporulation medium (humic acid)) after three days incubation in hot humid air.

## DISCUSSION

Control driven test method improvements and the use of multiple independent spore preparations with a single protocol useful for both *B. anthracis* Sterne and *B. thuringiensis* Al Hakam (Buhr et al 2012) allowed for the application of a statistically based experimental design, specifically RSM. This use of RSM analysis of test data for multiple combinations of spore strains, spore preparations, temperature, time, RH, materials, and debris permitted subsequent mathematical analysis and modeling of the response, generating a predictive capability valuable to potential end users of hot, humid air decontamination technology.

## ACKNOWLEDGEMENTS

This work was supported through funding provided by the Defense Threat Reduction Agency Joint Science and Technology Office, Protection and Hazard Mitigation Capability Area (Project Number BA08PHM113). Dr. Chuck Bass is credited with the recommendation to test kaolin. Dr. Martin Page is credited with the selection of humic acid. We thank our colleagues at the Naval Surface Warfare Center, Dahlgren Division and innumerable collaborators.

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Prokop, E.J., Crigler, J.R., Wells, C.M., Young, A.A., Buhr, T.L. (2014) Response Surface Modeling of Hot, humid air decontamination of materials contaminated with *Bacillus anthracis* ΔSterne and *B. thuringiensis* Al Hakam spores. AMB Express 4:21

T.L. Buhr, A.A. Young, H. Barnette, Z.A. Minter, N. Kennihan, C.A. Johnson, M. Bohmke, M. DePaola, M. Page. (2015) Test methods and response surface models for hot, humid air decontamination of materials contaminated with dirty spores of *Bacillus anthracis* ΔSterne and *B. thuringiensis* Al Hakam.

# New Developments in the Solid Oxidizer Decontamination Technology – Dahlgren Decon

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## Introduction

Peracetic acid is a well-known oxidizer with demonstrated success in oxidizing traditional chemical threat agents and inactivating bacterial spores/vegetative cells. Naval Surface Warfare Center Dahlgren Division (NSWCDD) previously developed a decontaminant technology which incorporates a solid peracid-containing borate salt, PES-Solid. This technology is called Dahlgren Decon. Unlike typical solid systems, the peracetic acid from PES-Solid is immediately available to neutralize threat agents and is readily soluble in water. Dahlgren Decon is safe, demonstrates excellent materials compatibility, and is user-friendly for the warfighter.

Dahlgren Decon, the formulation developed by NSWCDD Code Z21, was successfully demonstrated against traditional chemical and biological agents in the Hazard Mitigation, Materiel and Equipment Restoration (HaMMER) Advanced Technology Demonstration (ATD). This fixed formulation product also served as the government baseline technology for the Joint General Purpose Decontaminant Hardened Military Equipment (JGPD-HME) program. Additionally, the technology was modularized in the Joint Science and Technology Office's (JSTO) "Dial-A-Decon" program and successfully transitioned to the Joint Project Manager Protection's (JPM P) DFoS technology portfolio at the end of 2012 with a Technology Readiness Level (TRL) of 6.

The objective of the work presented in this poster was to spectroscopically identify the active species present in solutions of PES-Solid and then identify the chemical mechanism of decomposition through kinetic measurements. Understanding the chemistry of the species present in aqueous solutions of PES-Solid is vital to understanding potential formula modifications necessary to optimize pot life and performance.

## PES-Solid (Peracetyl Borate)

The structure proposed in Figure 1 contains peracetic acid (PAA), a powerful oxidant and a highly efficacious biocide, which can act as a general purpose chemical and biological decontaminant. Solid forms of PAA have been highly sought in order to improve safety and handling concerns with concentrated solutions. PES-Solid is an advantageous PAA source due to its high oxidant content and the instantaneous availability of active oxidant upon dissolution.

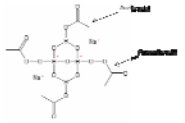

**Figure 1.** The proposed structure of PES-Solid.

**Table 1.** The formula, contributions of each element to the total formula weight, and the percent by weight composition of each element for the structure in Figure 1.

| Element | molar mass | Formula | Total | Percent |
|---|---|---|---|---|
| B | 10.81 | 4 | 43.24 | 9.89% |
| C | 12.011 | 8 | 96.088 | 21.97% |
| H | 1.008 | 12 | 12.096 | 2.77% |
| O | 15.999 | 15 | 239.985 | 54.87% |
| Na | 22.99 | 2 | 45.98 | 10.51% |
| | | Formula Wt.: | 437.389 | |

Theoretical weight% PAA based on the proposed structure: **34%**

## Spectroscopic Analysis of Aqueous PES-Solid Solutions

### FT-IR Spectroscopy

The ATR-FTIR spectrum for a solution of PES-Solid at pH 7 is shown in Figure 2. Spectra of aqueous solutions of PAA and a mixture of PAA and sodium tetraborate are also shown for comparison. The frequency of the C=O stretch of the acetic acid species is the same as that of sodium acetate, indicating that the AA is not complexed with boron at this pH. The C=O stretch of the PAA moiety is shifted to a slightly lower frequency in the PES-Solid/PAA-tetraborate mix spectra than what is observed in the PAA solution at the same pH. This shift is evidence of chemical attachment of PAA to a borate species in solution.

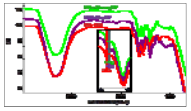

**Figure 2.** FT-IR data for solutions of PAA, PES-Solid, and a mixture of PAA and sodium tetraborate.

### NMR Spectroscopy

Proton ($^1$H), carbon-13 ($^{13}$C), and boron-11 ($^{11}$B) Nuclear Magnetic Resonance (NMR) spectroscopy conducted on aqueous solutions of PES-Solid revealed some additional information about the possible molecular structure in solution. A series of $^1$H and $^{13}$C spectra comparing solutions of PAA and of PES-Solid at a series of pH values between 5 and 9 are in Figures 3 and 4. Examination of this data indicates that a likely PAA deprotonation event occurs in solutions of PAA and not in solutions of PES-Solid. A series of $^{11}$B spectra for the solutions of PES-Solid are also shown in Figure 5.

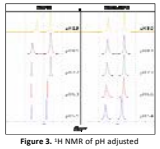

**Figure 3.** $^1$H NMR of pH adjusted solutions of PAA and PES-Solid.

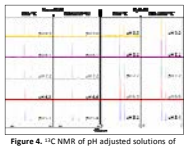

**Figure 4.** $^{13}$C NMR of pH adjusted solutions of PAA and PES-Solid.

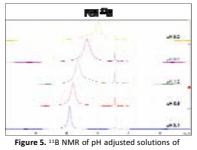

**Figure 5.** $^{11}$B NMR of pH adjusted solutions of PES-Solid.

### Proposed Structure in Solution

Both the IR and NMR data point to the existence of a PAA-boron complex in aqueous solutions of PES-Solid. The nature of the complex and the mole fraction of PAA that it accounts for is less certain. In principle, the PAA can attach to either the trigonal or tetrahedral species shown in Figure 6A or 6B respectively. Current $^{11}$B NMR data implies that attachment to structures of type A is strongly favored, in concurrence with results obtained for other oxygen ligands.[1]

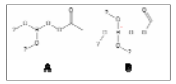

**Figure 6.** Proposed structures of the PAA species in solutions of PES-Solid.

## PAA Decomposition Rates

In a practical sense, understanding the evolution of the oxidant concentrations in aqueous solutions of PES-Solid over time indicates how long a particular decontamination solution is expected to be effective. However, information on the chemical mechanism through which PAA degrades is available when observing the degradation under strictly controlled conditions. In other words, a precise understanding of the degradation kinetics will provide insight into the chemical state of the oxidant which is available for reaction both through these decomposition reactions as well as in reactions with chemical threat agents.

The degradation of PAA in aqueous solutions can potentially occur through the multiple pathways shown in Scheme 1A-C.[2,3] Pathway A is termed spontaneous decomposition and involves nucleophilic attack of the deprotonated form of PAA at the carbonyl of the neutral acid. Because both the acid and its anion are involved in the reaction, the rate for this pathway reaches a maximum at the $pK_a$ of PAA and proceeds through second order kinetics. In pathway B, the PAA is hydrolyzed by nucleophilic attack at the carbonyl and since this reaction involves hydroxide anion, an increase in pH results in an increase in the rate of loss of PAA. In pathway C, the decomposition of PAA is accelerated through coordination with a transition metal, followed by rapid decomposition of the complex.

Spectroscopic data suggests that the peracetic acid found in aqueous solutions of PES-Solid is present as a borate complex. Therefore, the PAA in those solutions would likely degrade via a different route. Two potential routes, D and E, are presented in Scheme 1. Pathway D is analogous to pathway B for free PAA. Nucleophilic attack at the peracid carboxyl group results in free acetic acid and a peroxy borate species. In pathway E, a peroxoborate species nucleophilically attacks the carbonyl of a borate complexed PAA. The resulting adduct decomposes to acetate and oxygen in a manner analogous to pathway A. Notably absent from the potential pathways of degradation of the borate-PAA complex is a spontaneous decomposition analog.

Figures 7 and 8 contain plots showing the concentration of PAA versus time in solutions of PAA diluted from concentrate and PES-Solid at pH 7, 8, and 9. A cursory evaluation of the data indicates that the trend in the decay rate with respect to pH is different between the two solutions, suggesting the PAA degrades through a different mechanism in the two solutions. However, further analysis of the data is required.

**Scheme 1.** Potential reaction routes for the decomposition of PAA or a PAA/borate complex in solution.

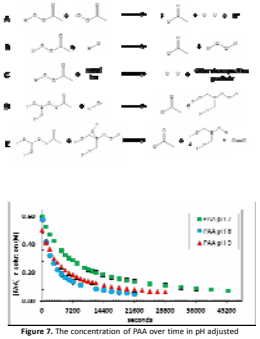

**Figure 7.** The concentration of PAA over time in pH adjusted solutions of PAA diluted from a commercial source.

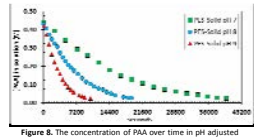

**Figure 8.** The concentration of PAA over time in pH adjusted solutions of PES-Solid.

## Evaluation of PAA Decomposition Kinetics

### Decomposition Kinetics Constants

Plots such as the ones in Figures 7 and 8 are useful when determining the "pot life" of a particular decon, however, further analysis of the data was needed in order to determine the fundamental processes of these reactions. Additional data analysis revealed the kinetics constants contained in the rate law equation (Eq. 1) for the reactions taking place. The reaction order, *n*, was determined using van't Hoff plots based on Eq. 2, in which the logarithm was taken of each side of Eq. 1. The slope of the line fit to the plot of log (-d[PAA]/dt) versus log [PAA] is equal to the order of the reaction. According to these plots the PAA solution decomposed via second order kinetics and the PES-Solid solution decomposed via first order kinetics (Figure 9). The results for the PAA solution matched with data found in the literature.[2,3]

The 1st and 2nd order integrated rate laws (Eq. 3 and Eq. 4) were used to derive the kinetics constants for each reaction. For second order reactions like the decomposition of PAA, the plot of 1/[PAA] vs. time should be a straight line with a slope equal to *k*. The plot of the ln[PAA] vs. time is equal to -*k* for first order reactions, like the decomposition of PAA in solutions of PES-Solid. The results of these plots are found in Figure 10.

**Eq. 1.** The rate law equation.

$$-\frac{d[AA]}{dt} = k[AA]^n$$

**Eq. 2.** The equation used for van't Hoff plots.

$$\log\left(-\frac{d[AA]}{dt}\right) = n \cdot \log[AA] + \log k$$

**Eq. 3.** 2nd order integrated rate law.

$$\frac{1}{[AA]} = k \cdot t + \frac{1}{[AA]_0}$$

**Eq. 4.** 1st order integrated rate law.

$$\ln[A] = -kt + \ln[A]_0$$

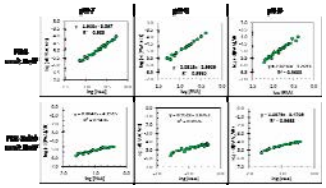

**Figure 9.** van't Hoff plots for the decomposition reactions shown in Figure 7 and Figure 8. The slope of these plots should be equal to the order, *n*, in the rate law equation.

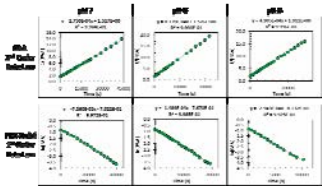

**Figure 10.** Integrated rate law plots for the decomposition reactions shown in Figure 7 and Figure 8. These plots should be linear with a slope equal to the rate constant, *k*, for 2nd order reactions, and -*k* for 1st order reactions.

As expected, the primary mode of degradation of PAA was through spontaneous decomposition (pathway A, Scheme 1). Spontaneous decomposition is highly pH dependent because it involves the reaction of a nucleophilic deprotonated PAA anion with an electrophilic neutral PAA molecule. The ratio of these two species in solution will be based on pH, and equivalent when the pH is equal to the $pK_a$ of PAA (8.2). That relationship led to the development of a method for determining the pH independent rate constant described in the literature by Yuan et al.[2,3] The method relates the value Q (Eq. 5) to the pH dependent (or observed) rate constants by plotting the observed rate constants versus the ratio $2Q/(1+Q)^2$. The result should be a straight line with a slope equal to the pH independent rate constant. The plot generated using the data in Table 2 results in a straight line with an $R^2$ of 0.996 (Figure 11). The pH independent rate constant was found to be $1.5 \times 10^{-3}$ L·mol$^{-1}$·s$^{-1}$, which is approximately 1.7 times lower than what was found in the literature ($2.6 \times 10^{-3}$ L·mol$^{-1}$·s$^{-1}$). This slight difference may be attributable to a lower transition metals contribution to the degradation rate in this work. The linearity of the plot and the match of the derived constant with the literature validates the system used in these experiments to derive the kinetics constants of PAA decomposition.

### Implications of the Decomposition Kinetics

Since the decomposition of PAA in solutions of PES-Solid is of a different reaction order, it is difficult to compare the reaction rates; however, comparisons of the 1st half-lives are possible using the same initial PAA concentration. The rate constants for the PES-Solid reactions are presented in Table 3 along with the rate constants for solutions of PAA. Also included in the table are half-lives based on the experimentally derived rate constant. For first order reactions, such as what was observed for PES-Solid, the half-life does not change with concentration. That is not true for a second order reaction, where the half-life is dependent on the starting concentration. Therefore, as the starting concentration of PAA increases, the length of the first half-life decreases. Table 3 compares the concentration independent half-life of the PAA in solutions of PES-Solid with the first half-life of PAA diluted from concentrate. The starting concentration was set equal to that of the starting concentration of PAA in Dahlgren Decon (0.64 M). At pH values of 7 and 8, the first half-life of PAA in solutions of PES-Solid is longer than it is for typical solutions of PAA.

**Table 2.** Information used to calculate the pH independent rate constant for PAA decomposition in aqueous solutions. These data match literature values well.

| pH | $[H^+]$ (M) | k observed ($M^{-1}s^{-1}$) | Q | $\frac{k_{obs}}{(\frac{2Q}{(1+Q)^2})}$ |
|---|---|---|---|---|
| 7.0 | $1 \times 10^{-7}$ | $2.8 \times 10^{-4}$ | 15.85 | 0.11 |
| 8.0 | $1 \times 10^{-8}$ | $8.1 \times 10^{-4}$ | 1.58 | 0.47 |
| 9.0 | $1 \times 10^{-9}$ | $4.9 \times 10^{-4}$ | 0.16 | 0.24 |

**Eq. 6.** Relationship of $k_{obs}$ to $k_{pH\ ind.}$ Based on Q.

$$\frac{k_{obs}}{\left(\frac{2Q}{(1+Q)^2}\right)} = k_{pH\ ind.}$$

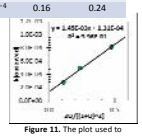

**Figure 11.** The plot used to calculate the pH independent rate constant. (reference 1)

**Table 3.** The observed rate constants for the decomposition of the PAA species in solutions of PES-Solid and PAA. Also presented is the length of the first half life assuming an initial concentration equal to that which is found in Dahlgren Decon.

| pH | PES-Solid Solution | | PAA Solution | |
|---|---|---|---|---|
| | k ($s^{-1}$) | $t_{1/2}$ (min) | | $t_{1/2}$ (min) $[PAA]_0 = 0.64M$ |
| 7.0 | $7.3 \times 10^{-5}$ | 158 | $2.8 \times 10^{-4}$ | 93 |
| 8.0 | $1.5 \times 10^{-4}$ | 77 | $8.1 \times 10^{-4}$ | 32 |
| 9.0 | $2.9 \times 10^{-4}$ | 40 | $4.9 \times 10^{-4}$ | 53 |

## Conclusions

As a result of work performed in this project, a clearer picture is emerging of the structure of PES-Solid, both in its solid form and in aqueous solution. The research reported here provides a deeper understanding of the degradation mechanism of PAA in PES-Solid solutions. The degradation of PAA in PES-Solid solutions was determined to be 1st order as compared to 2nd order for solutions of PAA prepared from concentrate. This finding complements spectroscopic evidence supporting the proposal that in aqueous solutions of PES-Solid, a significant proportion of the peracetic acid exists as a complex with the boric acid/borate salts. The existence of this proposed complex then alters the degradation pathway of PAA in these solutions. Consequently, the existence of a complex would likely impact the decontamination chemistry, but as evidenced from previous PES-Solid efficacy results, it does not negatively impact its reaction with threat agents.

In summary, a precise understanding of the degradation kinetics provides insight into the chemical state of the oxidant which is available for reaction, both through these decomposition reactions as well as in reactions with chemical threat agents. Ultimately, those insights help to understand the mode(s) of reaction with chemical agents and provide potential means of enhancing the efficacy of PES-Solid based decontamination formulations against a variety of threats agents.

### Future Work

Additional work in this area will focus on the impact of operational conditions on the kinetics of PAA decomposition in solutions of PES-Solid. These factors include: transition metal contamination, heat of mixing, decon additives, and batch size. Experiments have demonstrated that these factors do impact the decomposition rate of PAA in solutions of PES-Solid, but a more detailed study will identify the mechanism of their contribution.

## Acknowledgments

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3. Yuan, Z. Ni, Y., and Heiningen, A. *Can. J. Chem. Eng.* **1997**, *75*, 42.

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**Statement A: Approved for Public Release; Distribution is unlimited**
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# DAHLGREN DECON – A Solid Oxidizer Decontaminant

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## INTRODUCTION

Current decontamination solutions are based on oxidative chemistry in aqueous systems. To avoid transporting extra water, it is desirable to reduce the logistical footprint of decontaminants by identifying solids to be mixed on site. One of the more challenging components is the oxidizing agent. While currently fielded high test hypochlorite (HTH) is a solid, it is also a harsh, halogenated material with poor materials compatibility. Non-halogenated peroxygen compounds are of interest as oxidizers because of their low impact on the environment and their relatively low toxicity. PES-Solid, made by Solvay Chemicals Inc., is a solid peracid-containing borate salt that provides 25-30 wt% peracetic acid (PAA) when dissolved in water. Peracetic acid is therefore immediately available for reaction with threat agents and is neither delayed by nor dependent upon the kinetics of *in situ* generation. Dahlgren Decon, a Navy patented decontaminant formulation incorporating PES-Solid in a surfactant blend, has been shown to provide improved decontamination efficacy against both biological and traditional chemical agents, improved materials compatibility and it offers the desired reduced logistical footprint. Dahlgren Decon was successfully evaluated as part of the Defense Threat Reduction Agency (DTRA) Hazard Mitigation, Materiel and Equipment Restoration Advanced Technology Demonstration (HaMMER ATD) and was used as the government baseline in the Joint Project Manager for Protection (JPM P) Joint General Purpose Decontaminant for Hardened Military Equipment (JGPD-HME) Competitive Prototype testing.

During previous developmental efforts at Naval Surface Warfare Center Dahlgren Division (NSWCDD), emphasis was placed on identifying a solid source of peracetic acid (PAA). Typical systems generate the peracid in situ upon mixing in water. For example, PAA can be generated from the reaction of hydrogen peroxide and tetraacetylethylenediamine[1]. This approach is feasible, but offers limited success partially because of poor solubility and a slowed reaction rate. A milestone was reached when an NSWCDD research team identified and successfully tested a novel, solid source of PAA originally developed for the commercial detergent industry. This peracetyl borate compound, trade name PES-Solid, does not depend on in situ generation of the peracid. The dissolution in water provides PAA availability for threat agent neutralization much more rapidly than in situ methods[1]. In conjunction with the above efforts, the NSWCDD research team developed, optimized and tested a microemulsion system to be used with PES-Solid to provide a complete decontaminant package. Appropriate selection of the surfactants and emulsion components ensured that the decontaminant remained physically and chemically stable over a broad temperature range and reasonable period of time. The total research effort resulted in the patented formulation of a Solid Oxidizer Decontaminant named **Dahlgren Decon**. In a number of laboratory test efforts, Dahlgren Decon has shown improved threat agent decontamination over currently fielded decontaminants. In addition, Dahlgren Decon provides improved materials compatibility and a reduction in logistical footprint. Follow on developmental work on Dahlgren Decon and the solid oxidizer technology determined the modularity of the formulation. Dahlgren Decon component ratios can be adjusted as needed to improve decontamination efficacy against target threat agents and/or save on component costs without negatively affecting the stability of the product. Dahlgren Decon is a safe and user friendly product reducing the risk and workload of the operators in the field.

1. Hodge, R.C.; Lawson, G.E.; Brown, J.S. Research and Development of the Surfactant-Based Chemical and Biological Decontaminating Solution (Dahlgren Decon). NSWCDD/TR-06/32. Dahlgren, VA, May 2006; unclassified report.

## DAHLGREN DECON MICROEMULSION

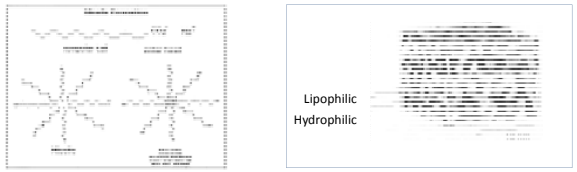



**Figure 1.** Microemulsion solution - surfactant system emulsifying oil soluble threat agents

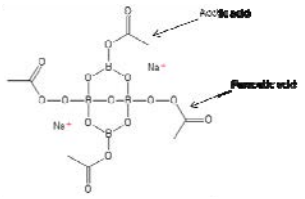



**Figure 2.** The proposed structure of PES-Solid.

## DAHLGREN DECON CWA EFFICACY

| Decon Suite | Material | HAMMER-E8-03 VX (Dahlgren Decon) (Vertical) Contact Hazard (mg/m²) | Runoff (mg/m²) | Splash (mg/m²) | HAMMER-E8-09 VX (Dalgren Decon) (Horizontal) Contact Hazard (mg/m²) | Runoff (mg/m²) | Splash (mg/m²) |
|---|---|---|---|---|---|---|---|
| MoM | CARC(W) | < 0.24 | < 0.24 | < 0.24 | < 0.24 | < 0.24 | < 0.24 |
| MoM | MoM | < 0.24 | < 0.24 | < 0.24 | < 0.24 | < 0.24 | < 0.24 |
| MoM | SBR | 30.70 (12.88 - 47.93) | 1.5 / 0.6 | 1.4 / 1.6 | 24.37 (20.95 - 28.36) | 2.9 / 0.4 | < 0.24 |
| Stationary | CARC(W) | < 0.24 | < 0.24 | < 0.24 | < 0.24 | < 0.24 | < 0.24 |
| Stationary | CARC(S) | < 0.24 | < 0.24 | 0.2 / 0.3 | < 0.24 | < 0.24 | < 0.24 |
| Stationary | SBR | 16.85 (13.39 - 21.21) | <0.24 | 2.5 / 0.3 | 12.35 (10.49 - 14.55) | 0.7 / 0.2 | < 0.24 |

| Decon Suite | Material | HAMMER-E8-01 VX (SuperSoap, Water, and AD/DA) (No Decons) (Vertical) Contact Hazard (mg/m²) | Runoff (mg/m²) | Splash (mg/m²) |
|---|---|---|---|---|
| SuperSoap 5 Min | CARC(W) | 1.16 (0.62-2.18) | 2.1 / 2.2 | 2.1 / 1.7 |
| SuperSoap 5 Min | CARC(S) | 1.7 (1.55 - 1.86) | 1.6 / 1.9 | 1.7 / 1.6 |
| SuperSoap 5 Min | SBR | 49.07 (33.10 - 72.76) | 1.2 / 1.6 | 1.3 / 0.6 |
| SuperSoap 30 Min | CARC(W) | 1.70 (1.51 – 1.90) | 1.4 / 1.9 | 1.3 / 0.5 |
| SuperSoap 30 Min | CARC(S) | 1.02 (0.78 – 1.33) | 0.7 / 0.8 | 0.5 / 0.2 |
| SuperSoap 30 Min | SBR | 59.13 (55.5 – 63.0) | 14 / 37 | 36 / 0.4 |

| Decon Suite | Material | HAMMER-E8-04 HD (Dahlgren Decon) (Vertical) Contact Hazard (mg/m²) | Runoff (mg/m²) | Splash (mg/m²) | HAMMER-E8-06 HD (Dahlgren Decon) (Horizontal) Contact Hazard (mg/m²) | Runoff (mg/m²) | Splash (mg/m²) |
|---|---|---|---|---|---|---|---|
| MoM | CARC(W) | 13.10 (2.30 - 74.54) | < 1.20 | < 1.20 | 2.36 (2.79 - 5.60) | < 1.20 | < 1.20 |
| MoM | CARC(S) | 7.62 (1.72 - 33.83) | < 1.20 | < 1.20 | 0.98 (0.70 - 2.01) | < 1.20 | < 1.20 |
| MoM | SBR | 197.77 (114.74 - 340.87) | 24 / 70 | 2.3 / 1.5 | 126.91 (114.18 - 141.06) | 8 / 1.8 | < 1.20 |
| Stationary | CARC(W) | 8.41 (2.03 - 34.77) | < 1.20 | < 1.20 | 1.21 (1.10 - 2.76) | < 1.20 | < 1.20 |
| Stationary | CARC(S) | 11.76 (6.11 - 22.65) | < 1.20 | < 1.20 | 1.12 (1.15 - 4.48) | < 1.20 | < 1.20 |
| Stationary | SBR | 220.06 (164.12 - 295.08) | 50 / 7.6 | 5.5/1.2 | 158.31 (122.62 – 187.64) | 5.5 / 4.4 | 2.2 / 11 |

| Decon Suite | Material | HAMMER-E8-01 HD (SuperSoap, Water, and AD/DA) (No Decons) (Vertical) Contact Hazard (mg/m²) | Runoff (mg/m²) | Splash (mg/m²) |
|---|---|---|---|---|
| SuperSoap 5 Min | CARC(W) | 11.83 (9.18-15.23) | 2.7 / 1.2 | 1.3 / 1.8 |
| SuperSoap 5 Min | CARC(S) | 6.27 (4.04-9.74) | 8.6 / 1.2 | 1.2 / 1.5 |
| SuperSoap 5 Min | SBR | 271.74 (219-309) | 4.0 / 2.4 | 5.2 / 20 |
| SuperSoap 30 Min | CARC(W) | 1.83 (1.32-3.14) | 4.8 / 2.5 | 1.2 / 1.2 |
| SuperSoap 30 Min | CARC(S) | 8.59 (6.30-14.6) | 2.7 / 1.2 | 1.2 / 1.2 |
| SuperSoap 30 Min | SBR | 149.76 (132-191) | 2.7 / 1.6 | 1.7 / 1.8 |

Threshold – VX: 0.78mg/m² HD: 100mg/m² Objective – VX: 0.3mg/m² HD: 10mg/m²

**Figure 1 .** Results from the DTRA HaMMER ATD large panel efficacy studies conducted at Battelle, Inc. The application of Dahlgren Decon in the two decon suite simulation studies resulted in attainment of the VX objective level for contact hazard on both CARC(W) and CARC(S) in either panel orientation, as well as the HD objective level in the horizontal position. The HD threshold level was attained in the vertical panel orientation for both CARC(W) and CARC(S).

## DAHLGREN DECON BWA EFFICACY

$Log_{10}$ survival of *B. anthracis* Ames, *B. anthracis* Δsterne, B. *thuringiensis* Al Hakam spores, *F. philomiragia* cells , and MS2 bacteriophage on seven different substrates after a 15-minute treatment with 50 g/L PES-Solid in two different solutions.

| | *B. anthracis* Ames (7.0±0.3 $log_{10}$/coupon) | *B. anthracis* ΔSterne (7.2 ±0.2 $log_{10}$/coupon) | *B. thuringiensis* Al Hakam (7.2 ±0.3 $log_{10}$/coupon) | *F. Philomiragia* (7.6 ±0.2 $log_{10}$/coupon) | MS2 (6.8 ±0.2 $log_{10}$/coupon) |
|---|---|---|---|---|---|
| **50 g/L PES-Solid in Water:** | | | | | |
| *CARC-W* | 1.3±0.8 | 1.7±1.3 | 1.3±1.6 | 1.3±1.7 | 0.6±1.3 |
| *MgF2 Glass* | 0.0±0 | *0.4±0.8 | 0.0±0 | 0.0±0.0 | 0.0±0.0 |
| *Stainless Steel 304* | *0.3±0.8 | 0.0±0 | 0.0±0 | 0.3±0.4 | 0.3±0.8 |
| *APC* | 0.0±0 | 0.0±0 | 0.0±0 | 0.2±0.2 | 0.7±1.5 |
| *NTC* | *0.4±0.9 | *0.1±0.3 | *0.1±0.2 | 0.7±0.9 | 0.0±0.0 |
| *Lexan* | 0.0±0 | 0.0±0 | 0.2±0.2 | 0.2±0.2 | 0.2±0.5 |
| *LDPE* | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.8±0.8 |
| **50 g/L PES-Solid in Dahlgren Surfactant System:** | | | | | |
| *CARC-W* | 0.3±0.6 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.0±0.0 |
| *MgF2 Glass* | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.0±0.0 |
| *Stainless Steel 304* | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.0±0.0 |
| *APC* | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.0±0.0 |
| *NTC* | 0.0±0 | *0.6±1.3 | 0.7±0.5 | 0.0±0.0 | 0.0±0.0 |
| *Lexan* | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.2±0.5 |
| *LDPE* | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0.0 | 0.0±0.0 |

*4 of 5 independent samples were 0.0 CFU while 1 of 5 independent samples had some spore survival. Spore extraction data not shown.

## DAHLGREN DECON MATERIALS COMPATIBILITY

Testing conducted under "worst case" conditions, employing a 24-hour immersion of the test material in Dahlgren Decon. Recommended TTPs will implement a 15-30 minute contact time followed by a water rinse.

| CLASS | MATERIAL | $H_2O$ CONTROL | DAHLGREN DECON | TEST PERFORMED | TEST METHOD | COMMENTS |
|---|---|---|---|---|---|---|
| **Coatings** | *CARC-S* | 0 | 0 | Coating Hardness (Δ pencil class) | ASTM D3363-92a, *Film Hardness by Pencil Test* | Results indicate the change in scratch/gouge rating after 24-hr immersion. A change of 2 pencil classes suggests alteration to the coating. |
| | *CARC-W* | 0 | -2 | | | |
| | *Non-Skid Type I* | 0 | 0 | | | |
| | *Non-Skid Type XI* | 0 | -2 | | | |
| **Elastomers** | *BUNA-N* | 0.08 | 0.25 | Sorption (% mass change) | ASTM D471-98, *Rubber Property - Effect of Liquids* | Sorption change of > 5% generally considered cause for concern or recommended change in TTPs. |
| | | -0.69 | -2.71 | Durometer Hardness (% change) | | |
| | *SBR* | 0.15 | 0.20 | S | | Durometer hardness test method failure criteria is stated as >2% change. This value is generally agreed to be too stringent as variability between labs is >8% and flexible material variability is itself typically ± 2%. |
| | | 1.78 | -5.21 | DH | | |
| | *Silicone Rubber* | -0.17 | -0.65 | S | | |
| | | 0.14 | -2.00 | DH | | |
| **Plastics** | *LEXAN* | 0.05 | 0.05 | S | ASTM D2240-97, *Rubber Property – Durometer Hardness.* | |
| | | -5.9 | -0.81 | DH | | |
| | | 53.23 | 316.13 | Haze, (% change) | ASTM D1003-97: *Haze and Luminous Transmittance of Transparent Plastics* | Cause for concern: > 500% change |
| | | -0.49 | 0.99 | Transmittance, (% change) | | Cause for concern: > 1% change |
| **Metals** | *4140 Steel Alloy* | -0.50 | 0.84 | Corrosion, (mpy) | ASTM standard G31-72(1995)ε1, *Laboratory Immersion Corrosion Testing of Metals.* | Corrosion Guidance<br>• < 2 mpy excellent<br>• 2-10 mpy good<br>• 10-20 mpy fair<br>• 20-50 mpy poor<br>• > 50 mpy severe |
| | *1020 Carbon Steel* | -1.25 | 0.18 | C | | |
| | *Brass, C2600* | 0.13 | -134.88 | C | | |

**Distribution Statement A: Approved for Public Release, Distribution is Unlimited**
**NSWCDD-PN-15-00238**

C-601

## ACKNOWLEDGEMENTS

Dahlgren Decon and Solid Oxidizer technology developmental work, biological efficacy and materials compatibility work was supported by the Defense Threat Reduction Agency (DTRA) and executed under the management of NSWCDD Z21. Chemical efficacy data from the HaMMER ATD was carried out at Battelle, Inc. and previously supplied by DTRA. We wish to thank our colleagues at DTRA, Battelle Inc. and our contributing colleagues at NSWCDD.

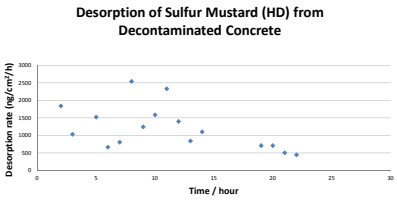
# How clean is safe? The detection of chemical warfare agent at ultra-low concentration after decontamination

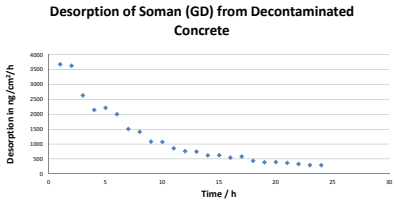
Chan Lai San Clareene, Chew Khee Siah Kendrick, Chia Chan Wing Andrew, Chua Hoe Chee and Loh Wai Leng

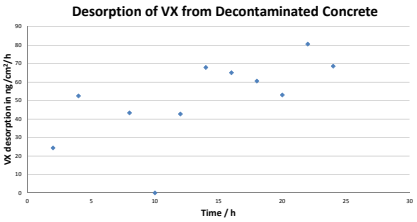
**DSO National Laboratories, 20 Science Park Drive, Singapore 118230**

## Introduction

- CWA can persist in environment for a long time.
- Porous surfaces (e.g. concrete) can trap CWA where decontaminants is unable to reach. Trapped CWA off-gas to pose a persistent desorption hazard.



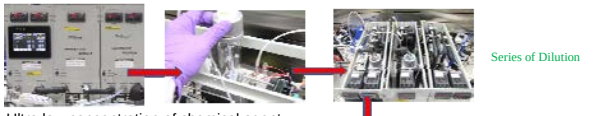

| Chemical Agent | Detection Limit of Chemical Detector (mg/m3) | | Desorption rate after 20 hours (ng/cm2/h) | Resultant Vapour Concentration in air (mg/m3) | Worker's Population Limit, WPL (mg/m3) |
|---|---|---|---|---|---|
| VX | Raid-M | 0.04 | 68 | 0.00113 (AEGL 2) | 0.000001 |
| | AP2C | 0.03 | | | |
| GD | Raid-M | 0.04 | 294 | 0.0049 (AEGL 2) | 0.00003 |
| | AP2C | 0.03 | | | |
| HD | Raid-M | 0.13 | 500 | 0.0083 (AEGL 1) | 0.0004 |
| | AP2C | 0.93 | | | |

Acute Exposure Guideline Levels (AEGL) are derived based on a 8 hours exposure.
For AEGL 2, it is predicted that above this concentration level, the public could experience irreversible or other serious, long lasting adverse health effects.

- Difficult if not impossible to remove and decontaminate CWA from surfaces, especially porous matrices.
- Decontaminant is unable to reach and detoxify agent that penetrated into a surface.
- Agent contaminated surfaces (even after decontamination) might continue to pose desorption hazard.
- Affect the re-occupancy of a contaminated site.
- Current CWA detectors capability is not sensitive enough to elucidate down to the level that is deemed to be safe for human occupancy.
- Worker Population Limit is the concentration at which an unprotected worker can operate safely 8 hours a day, 5 days a week for a working lifetime without adverse health effect.
- As desorption hazard is above WPL and current detector detection limit is above desorption level, it is imperative to develop a methodology that is able to quantitate agents at ultra-low concentration.

## Materials and Methods

Set Conditions | Insert Permeation Tubes



Series of Dilution

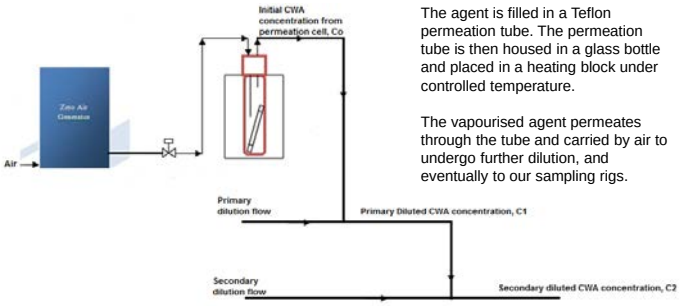
Sampling | Quantification using ATD-GCMS

Ultra low concentration of chemical agent vapour is generated using vapour generation system.

This system consists of at least a permeation oven and a dilution unit to produce the desired WPL concentration.

Air samples are collected using air sampling tube packed with adsorbent, Tenax TA.

For HD and GB, the air samples collected were analysed and quantified using an Auto Thermal Desorber coupled with Gas Chromatography Mass Spectrometer (ATD-GCMS).



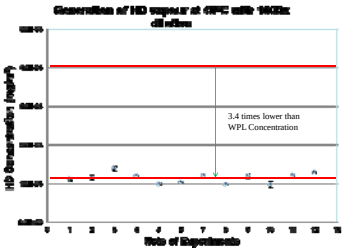

The agent is filled in a Teflon permeation tube. The permeation tube is then housed in a glass bottle and placed in a heating block under controlled temperature.

The vapourised agent permeates through the tube and carried by air to undergo further dilution, and eventually to our sampling rigs.

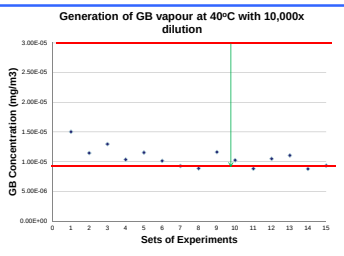
## Results

We have successfully generated, identified and quantitated Sulfur Mustard (HD) and Sarin (GB) vapour at their sub-WPL level.

Average HD concentration is at 1.15 x $10^{-4}$ mg/m3 with standard deviation of less than 12% over the sets of experiments.




Average GB concentration is at 9.80 x $10^{-6}$ mg/$m^3$ with standard deviation of less than 12% over the sets of experiments.




## Upcoming Work

With the robust result obtained for the 2 agents, it gives us the confidence to extend the validation methodology to other chemical agents. This can be achieved by:

1. To study the vapour generation of other chemical agents at their ultra-low concentration (WPL).
2. To explore other vapour generation techniques.
3. To identify and quantify the vapour generated by developing analytical methodology using suitable analytical instrument configurations (GC-MS-MS, GC-FPD, LCMS, etc).

## Conclusion

DSO has developed the capability to generate a steady and sustainable flow of ultra-low concentrations of agent HD and GB, and aims to do so for other agents. This will enable the validation of laboratory methods to sample and quantify agents at their sub-WPL levels.

This capability is essential for authorities to annunciate a chemical agent incident area back to normalcy, with high degree of confidence.

# Facility Decontamination Strategy and Technology Selection Tool

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S. Ryan‡, P. Lemieux‡, Leroy Mickelsen‡, Mario Ierardi‡

†Sandia National Laboratories, ††Sandia National Laboratories (retired),‡U.S. Environmental Protection Agency

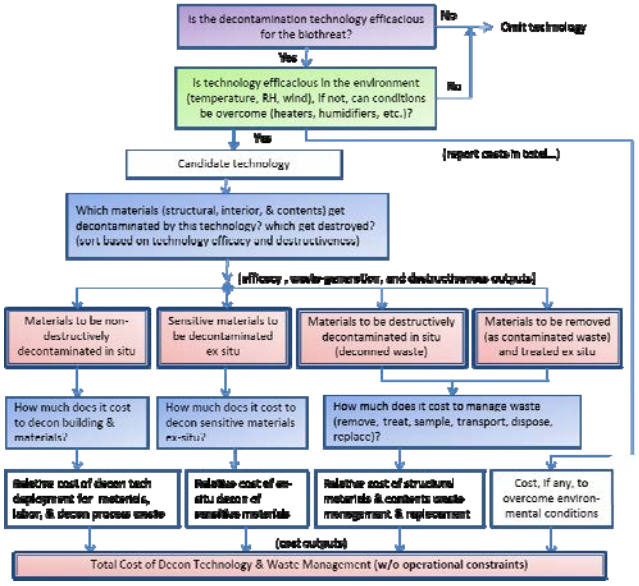
## Problem

**Facility remediation following *B. anthracis* contamination is a complex problem.**

For each potential decontamination technology, decision makers must balance consideration of the performance data on each of the facility materials (structural, interior, and contents), the cost of the decontamination process, availability of resources, time required, the destructiveness and waste generated.

## Approach

**Create a comprehensive tool – the DeconST – that supports the decision process, by combining the IBRD-developed Decon Trade-Off tool with EPA's Incident Waste Decision Support Tool (I-WASTE DST) plus published, scientific literature on decontamination technologies.**




## Results

**For each decontamination technology applied to a specific facility, the DeconST shows the efficacy, destructiveness, and waste generated, as well as the total relative cost of the complete decontamination process, including waste handling.**

Furthermore, the DeconST

- Considers the particular facility structural and interior materials as well as the building contents
- Highlights special considerations that might affect the results (e.g., HVAC accessibility)
- Is not an expert system, but instead compares the estimated viability of all available options without removing any from consideration


## Impact

**The DeconST has been**

- Formally transferred from DHS-S&T to USEPA
- Written into the draft USEPA Operational Bio Guide for the USEPA responders, the likely users being the Technical Working Group providing input to the Incident Command
- Integrated by the DoD DTRA's Transatlantic Collaborative Biological Resiliency Demonstration (TaCBRD) program into its TaCBoaRD integrated suite of response and recovery decision-support tools

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SAND2015-2899C

# Aerosol Delivery of Liquid Decontaminants: A Novel Approach for Decontamination of Complex Interior Spaces

Sandia National Laboratories, Albuquerque, NM USA
Mark D. Tucker, Ph.D., Andres Sanchez, Joshua Hubbard, Ph.D., Matthew Tezak, Matthew Hankins, Ph.D., Scott Davison, Ph.D., Steven Storch, Brandon Servantes

## Problem:

Decontamination of *Bacillus anthracis* spores and other persistent agents (e.g. Sulfur Mustard [HD]) in critical infrastructure (e.g., subway systems, major airports) and critical assets (e.g., the interior of aircraft) can be challenging because effective decontaminants can damage materials.

Current decontamination methods require the use of highly toxic and/or highly corrosive chemical solutions because bacterial spores are very difficult to kill and other agents may require oxidation.

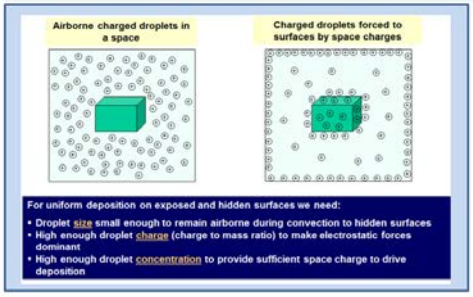
Complex deployment system for chlorine dioxide gas inside of a contaminated facility following the 2001 anthrax attacks.

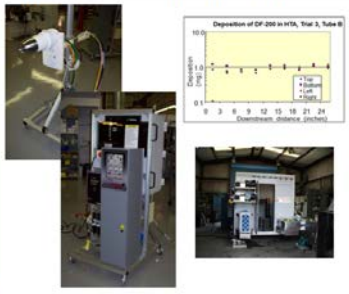
Corrosion of a metal lamp fixture following application of chlorine dioxide gas inside of an office building.

Photos courtesy of US EPA

## Concept:

Sandia (in collaboration with private industry) has developed a unique process to disperse aqueous and non-aqueous liquid decontaminants nearly uniformly on all surfaces in a space via charged aerosols.

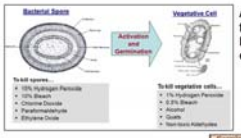


## Approach #1 (Germination):

We have developed a non-toxic, non-corrosive decon method to kill highly resistant bacterial spores.

- A chemical solution that triggers the germination process in bacterial spores to cause them to rapidly and completely change to much less-resistant vegetative cells which can be easily killed.
- Vegetative cells are then exposed to mild chemicals (e.g., low concentrations of hydrogen peroxide, quaternary ammonium compounds, alcohols, aldehydes, etc.) or natural elements (e.g., heat, humidity, ultraviolet light, etc.) for complete and rapid kill.




Aggressive fumigation formulations are currently needed because bacterial spores are extremely resistant.

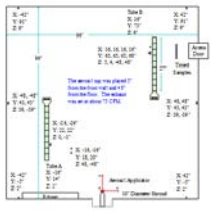
Kill of *Bacillus cereus* spores (an anthrax surrogate) with and without the addition of a germination solution (CFU's = colony forming units).

| Germination Solution (GS) | Kill Solution (KS) | Time Exposed GS | Time Exposed KS | Log CFU's Remaining |
|---|---|---|---|---|
| PBS (Control) | None | - | - | 6.27 |
| None | 3% $H_2O_2$ | - | 60 min | 6.41 |
| None | 6% $H_2O_2$ | - | 60 min | 6.42 |
| 5% of GS Mix in DI $H_2O$ | 3% $H_2O_2$ | 60 min | 60 min | 2.55 |
| 10% of GS Mix in DI $H_2O$ | 3% $H_2O_2$ | 60 min | 60 min | 2.22 |
| 15% of GS Mix in DI $H_2O$ | 3% $H_2O_2$ | 60 min | 60 min | 1.99 |
| 20% of GS Mix in DI $H_2O$ | 3% $H_2O_2$ | 60 min | 60 min | 1.59 |
| 25% of GS Mix in DI $H_2O$ | 3% $H_2O_2$ | 60 min | 60 min | 0 |
| 30% of GS Mix in DI $H_2O$ | 3% $H_2O_2$ | 60 min | 60 min | 0 |

Both the germination and kill solutions are delivered to the enclosed space via charged aerosols

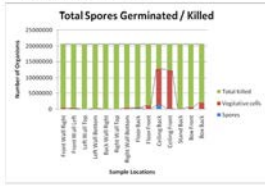
## Germination Results:

Dispersal testing of the germination and kill solutions via charged aerosols resulted in ~2 x $10^7$ bacterial spores killed on coupons mounted in various locations in the test chamber.



Red = germinated spores
Blue = ungerminated spores

Green = spores that germinated and were killed
Red = spores that germinated and were not killed
Blue = ungerminated spores


## Approach #2 (Liquid Decontaminants):

Liquid decontaminants (DF-200 and peracetic acid) were deployed as charged aerosols against CBW agent simulants in the Sandia Test Chamber. High efficacy was achieved.

***Bacillus atrophaeus* spore decon with DF-200**

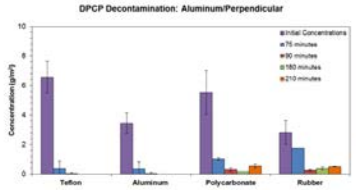
| Sample | Description | Bio-Efficacy Trial 38 | 39 | 40 |
|---|---|---|---|---|
| Process Data | | | | |
| | Charge (KV) | Level 1 | Level 2 | Level 3 |
| | Aerosol Concentration mg/m³ | 207.8 | 76.8 | 39.6 |
| Log CFU Results | | | | |
| 47-56 | Controls (Avg. of 10) | 6.32 | 6.28 | 6.23 |
| 1-12 | Wall Samples | 0 | 0 | 0 |
| 13 | Top Sample, Stand A | 3.42 | 4.07 | 0 |
| 14 | Bottom Sample, Stand A | 0 | 2.64 | 0 |
| 15 | Top Sample, Stand B | 3.39 | 3.01 | 0 |
| 16 | Bottom Sample, Stand B | 5.14 | 3.31 | 0 |
| 17 | Timed Sample (30 min) | 4.20 | 5.70 | 5.69 |
| 18 | Timed Sample (60 min) | 0 | 4.15 | 4.27 |
| 19 | Timed Sample (90 min) | 0 | 0 | 4.00 |
| 20 | Timed Sample (120 min) | 0 | 0 | 3.44 |
| 21 | Timed Sample (150 min) | 0 | 0 | 0 |
| 27-46 | Hamster Tube Samples | 0 | 0** | 0 |

*Colony Forming Units
**One out of 20 Hamster tube samples showed very mild growth.

**Bacillus atrophaeus spore decon with peractic acid**

| Large Coupon | Average Initial Concentration (CFU/coupon) | Average Initial Concentration (CFU/in²) | Post-Decon Concentration (CFU/coupon) | Log Kill per Coupon |
|---|---|---|---|---|
| Test 1 | $1.60 \times 10^8$ | $1.38 \times 10^6$ | 0 | 6.20 |
| Test 2 | $1.60 \times 10^8$ | $1.38 \times 10^6$ | 0 | 6.20 |
| Test 3 | $3.32 \times 10^8$ | $3.32 \times 10^6$ | 0 | 6.58 |

>6-Log Kill




CWA simulant (Diphenyl chlorophosphate) decon with DF-200

Exceeded JSSED/JPID Key Performance Parameter (KPP) objectives on all coupons except rubber

## Impact:

- A aerosol deployment device has been tested that can give nearly uniform coverage of liquid decontaminants on surfaces in interior spaces.
- Two approaches have been evaluated: Direct application of decontaminants and a two-step approach utilizing a germination solution.
- Preliminary methods have been tested that demonstrate high rates of decontamination of BW and CW surrogates.
- Not decontaminant specific.
- Could potentially be used for many types of complex spaces: Aircraft, subway cars, emergency vehicles, etc.
- Could potentially be used in conjunction with other processes (e.g., prior to hot, humid air decontamination).

Acknowledgements: Funding for this work was provided by Sandia National Laboratories LDRD (Laboratory Directed Research & Development) and by the U.S. Department of Defense, Defense Threat Reduction Agency (DTRA).

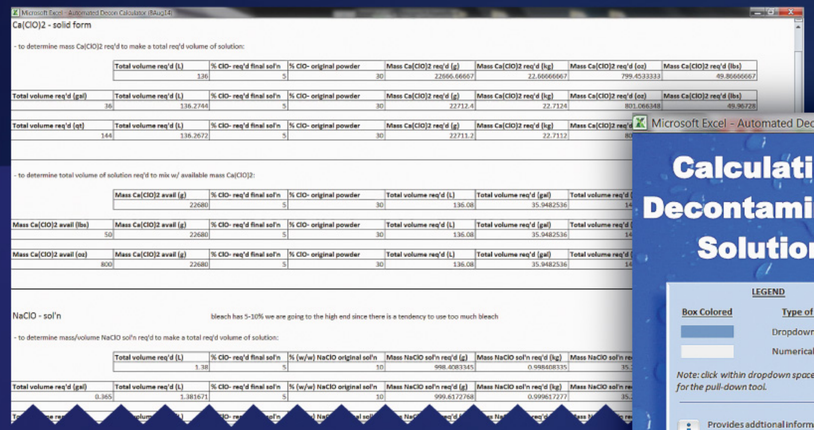


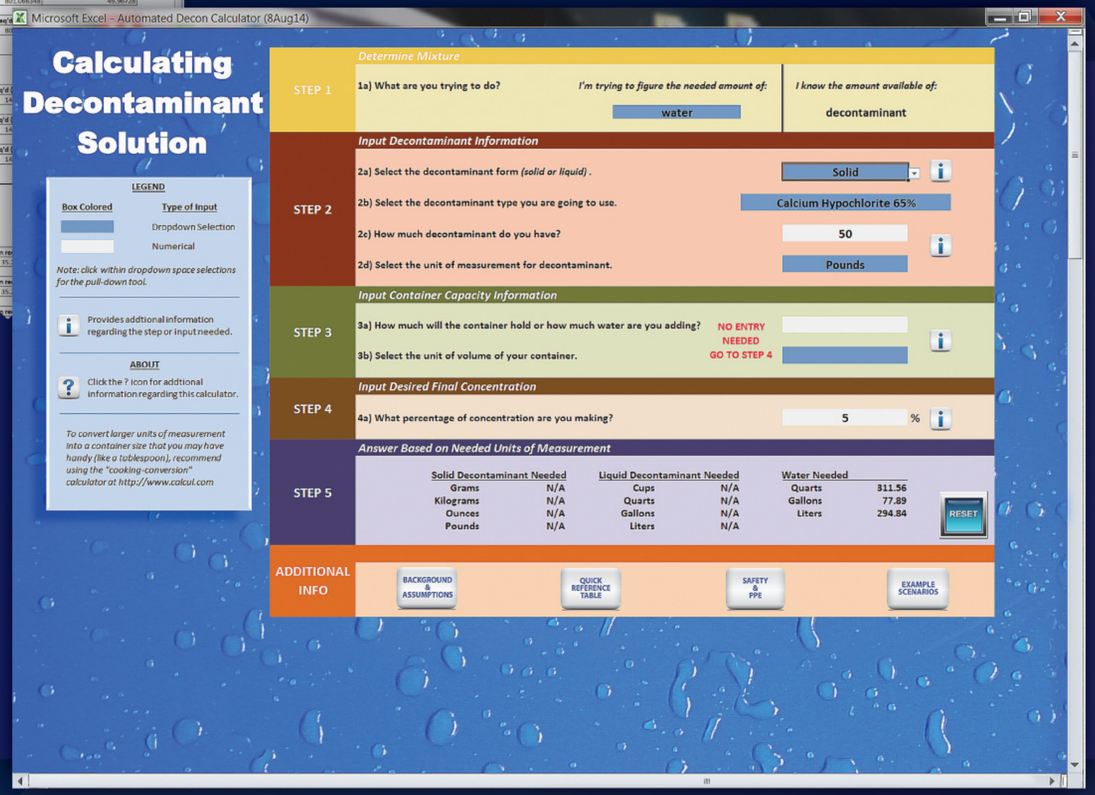


SAND2015-3048C

# AUTOMATED DECONTAMINATION CALCULATOR

**Provides the ability to make a chlorine-based decontamination solution at an appropriate concentration level without knowledge of chemistry principles.**




Formula calculations occur in the background to alleviate the guesswork; turning these complex chemical formulas into five simple steps.

Formulas allow for a variety of measurements (SI and English units).

Tool uses HTH, HTB, Calcium Hypochlorite (65% & 67%), and Sodium Hypochlorite (5%, 7%, & 10%).

Powered by Microsoft Excel 2010, the interactive interface provides:

- Four clear, concise, easy to answer questions
  - Natural step progression; allows user to achieve their goals
- A strong visual hierarchy
  - Allows the user to focus on what is most important
- Easy access help icons and teaching scenarios
- Consistent and accurate required concentrations
- Achievement of immediate goals
  - Leads to user's increased confidence

Approved for use in three U.S. Multi-Service Publications and U.S. Army Medical Research Institute of Chemical Defense (USAMRICD)



Air Force Civil Engineer Center
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C-605

# Enhanced Isolation of Viable *Bacillus* Spores Using Commercially Available Cell Lysis Solutions

Douglas W. Hamilton[1], Erin Silvestri[2] and Paul Lemieux[3]

[1]ORISE Research Participant • [2,3]United States Environmental Protection Agency • [2,3]National Homeland Security Research Center

## Abstract

The response to the intentional dissemination of *Bacillus anthracis* spores (anthrax) via the U.S. Postal Service in 2001, and subsequent research activities and planning exercises highlighted the extent to which different materials in a building might be contaminated. Subsequent research endeavors focused on the development of sample collection and analytical methods suitable for determining the efficacy of decontamination strategies and to characterize residual wastes. Sample collection methods for surfaces routinely employ swabs, wipes and vacuum socks with culture methods serving as the analytical "gold standard" for analysis[1]. Recovering spores from complex matrices (e.g., soils, porous building materials, and heterogeneous waste and debris) has been achieved with mixed results by mixing the sample with an aqueous carrier medium to generate a slurry that can be manipulated[2]. Quantification of spores from these slurries using culture methods can be challenging due to the concurrent growth of native organisms in the sample on culture media; therefore, sample processing methods capable of reducing background flora would enhance the analytical capabilities and improve the characterization of a sample.

Studies of coat proteins (*B. atrophaeus*) and exosporium proteins (*B. anthracis*, *B. thuringiensis*) of spore-forming bacteria have identified a possible strategy that may be useful in spore recovery and analysis from complex matrices. The efficient dissociation of spore exosporium proteins is typically realized only after treatment with strong denaturants (e.g., SDS buffer + 8M urea) and harsh physical treatment (e.g., boiling)[3]. In contrast, the "gentle" disruption of vegetative bacteria can be routinely achieved with commercially available lysis solutions. Standard protocols for these lysis solutions typically require short incubation times with buffer and are performed under ambient conditions, potentially allowing for high-throughput processing of multiple samples.

Currently, no information is available for the behavior of these commercial reagents with regard to spore inactivation. It is hypothesized that the hardy nature of the spore could be exploited, whereby the spore would remain viable under conditions that reduce the viability of vegetative bacteria. Specifically, chemical, physical and/or enzymatic treatment could be used to reduce, or eliminate, the presence of native vegetative organisms, thereby enhancing spore analytical procedures and improving sample characterization. The data presented herein summarize initial efforts in reducing the growth of vegetative *Escherichia coli* and *Enterococcus faecalis* using commercially available lysis solutions and characterizes the influence of these solutions on *B. atrophaeus* spore germination. Additionally, a comparison is presented between the spread plate technique and the spiral plate technique for the enumeration of spores and bacteria.

## Introduction

### Background:

EPA is designated as a coordinating Agency, under the National Response Framework, to prepare for, respond to and recover from a threat to public health, welfare or the environment. These threats include chemical, biological and radiological substances, whether accidentally or intentionally released. Following the terrorist events of 2001, EPA formed the National Homeland Security Research Center (NHSRC) in 2002 to perform research to address emergency response knowledge gaps. Part of NHSRC's mission is to develop and evaluate decontamination methods for contaminated indoor and outdoor areas as well as treatment and disposal methods for contaminated waste and debris. Research efforts are focused on cost-effective best practices that support decision making following homeland security incidents.

### Objectives:

- Identify sample processing methods capable of enhancing culture-based analytical methods for *Bacillus* spore characterization.
- Evaluate two sample preparation methods:
  1. Suspension in PBS
  2. Suspension in PBS + 0.05% Tween 20
- Evaluate two culture-based analytical methods:
  1. Spread Plate Method
  2. Spiral Plate Method
- Evaluate the ability of commercially available cell lysis solutions to reduce, or eliminate, the number vegetative organisms in a sample while preserving spore viability.

## Materials and Methods

**Sample Preparation & Analytical Methods**

| Organism | Suspension Solution | Analytical Method | Statistics |
|---|---|---|---|
| *B. atrophaeus* (spores)<br>*E. coli*<br>*E. faecalis* | PBS (n=5)<br>PBS + 0.05% Tween 20 (n=5) | Spread Plate<br>Spiral Plate | One-way AVONA<br>Tukey's Test<br>IBM SPSS Statistics v. 22 |

**Reduction of Vegetative Bacteria**

| Organism | Lysis Method | Analytical Method | Statistics |
|---|---|---|---|
| *B. atrophaeus* (spores)<br>*E. coli*<br>*E. faecalis* | B-PER (n=3)<br>B-PER + Lysozyme (n=3) | Spiral Plate | One-way AVONA<br>Tukey's Test<br>IBM SPSS Statistics v. 22 |

## Results

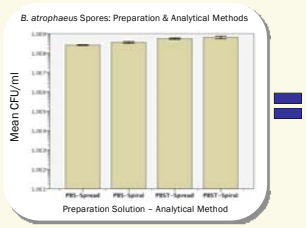
**Sample Preparation & Analytical Methods**

**ANOVA Summary**

| | *B. atrophaeus* Spores | *E. coli* | *E. faecalis* |
|---|---|---|---|
| Test of Normality (Shapiro-Wilk) | ✓ | ✓ | ✓ |
| Homogeneity of Variance (Levene Statistic) | ✓ | ✓ | ✓ |
| ANOVA Significance | <.000 | 0.001 | <.000 |

### Preparation Solution & Analytical Method Evaluation

(Error Bars = 95% Confidence Interval)

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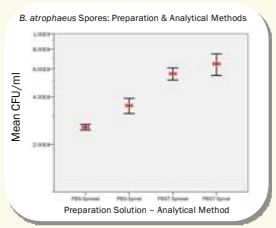
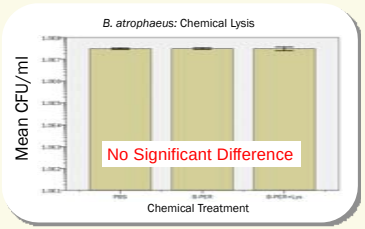


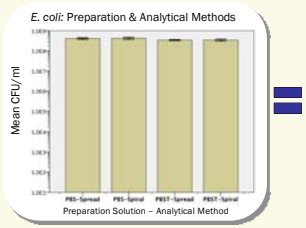

### Sample Comparison

(Tukey's Test, *p*-value)

| | PBS-Spiral | PBST-Spread | PBST-Spiral |
|---|---|---|---|
| PBS-Spread | 0.030 | 0.000 | 0.000 |
| PBS-Spiral | | 0.000 | 0.000 |
| PBST-Spread | | | 0.050 |

### Reduction of Vegetative Bacteria

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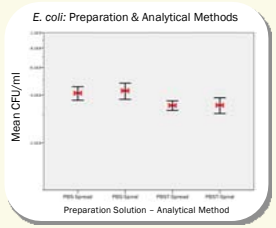
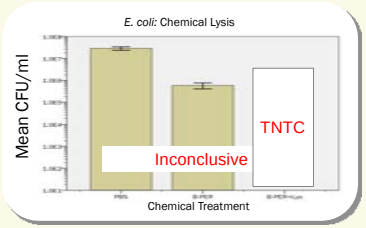
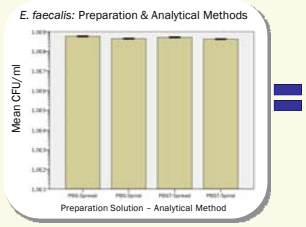



| | PBS-Spiral | PBST-Spread | PBST-Spiral |
|---|---|---|---|
| PBS-Spread | 0.885 | 0.019 | 0.020 |
| PBS-Spiral | | 0.004 | 0.005 |
| PBST-Spread | | | 1.000 |

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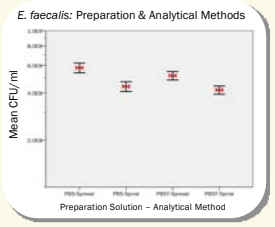
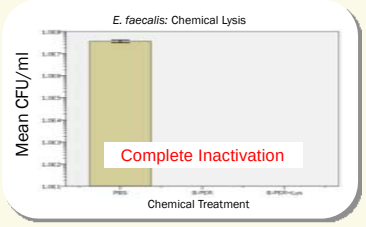



| | PBS-Spiral | PBST-Spread | PBST-Spiral |
|---|---|---|---|
| PBS-Spread | 0.000 | 0.009 | 0.000 |
| PBS-Spiral | | 0.002 | 0.557 |
| PBST-Spread | | | 0.000 |




## Conclusions

**Sample Preparation & Analytical Methods**

- Analysis of sample preparation methods using the *p*-value from the Tukey's Test indicated that PBST resulted in enhanced *Bacillus* spore dispersion, slightly reduced *E. coli* viability, and resulted in mixed results for *E. faecalis*.
- Comparison of the spread plate technique and the spiral plate technique resulted in mixed results.
- It was concluded that any combination of preparation methods is suitable for the purposes of this study. As such, the decision was made to conduct experiments by preparing sample spikes in PBST and analyzing samples by the spread plate method.

**Reduction of Vegetative Bacteria**

- It was determined that B-PER reagent and B-PER reagent amended with Lysozyme did not reduce the viability of *B. atrophaeus* spores.
- *E. faecalis* was completely inactivated by both reagent treatments, indicating that the standard protocol recommended by the manufacturer is effective for this organism.
- Treatment of *E. coli* produced mixed results with the manufacturer's standard protocol, and resulted in only minimal inactivation.
- Future experiments will be carried out using additional treatments in the presence of B-PER in an effort to achieve the targeted 6-$\log_{10}$ reduction in viability.

## 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 report 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.

## References

1. US EPA, *Selected analytical methods for environmental remediation and recovery (SAM)* - 2012. EPA/600/R-12/555, Washington, DC 2012.
2. Silvestri, E., Perkins, S., Feldhake, D., Nichols, T., and Schaefer, F., III, *Recent literature review of soil processing methods for recovery of Bacillus anthracis spores.* Annals of Microbiology, 2014: DOI 10.1007/s13213-014-0932-x.
3. Thompson, B.M., Binkley, J.M., and Stewart, G.C., *Current physical and SDS extraction methods do not efficiently remove exosporium proteins from Bacillus anthracis spores.* Journal of Microbiological Methods, 2011. 85(2): 143-148.

# Community Environmental Resilience Indicators

**Keely Maxwell, US Environmental Protection Agency (EPA)**

[1]Presidential Policy Directive (PPD)-21. 2013. Critical Infrastructure Security and Resilience, and Executive Order (E.O.) 13653. 2013. Preparing the United States for the Impacts of Climate Change. [2]EPA. 2014. EPA Explores Interest in Developing Community Environmental Resilience Indicators and Indices. Technical Brief. Washington, D.C.

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## A. What is community environmental resilience

**Resilience** is

*"the ability to anticipate, prepare for, and adapt to changing conditions and withstand, respond to, and recover rapidly from disruptions"*[1]

**Environmental resilience** is

*"Minimizing environmental risks associated with disasters, quickly returning critical environmental & ecological services to functionality after a disaster, while applying this learning process to reduce vulnerabilities & risks to future incidents."*[2]

### How can indicators help EPA support resilience?

Across the United States, communities experience earthquakes, extreme weather events, technological accidents, and other disruptive incidents. Disasters destroy critical infrastructure and natural resources, damage human health and the local economy, displace human populations, and disrupt environmental services. Federal policies that address disasters, homeland security, and climate change have begun to use resilience as a guiding principle. Resilience can help communities mitigate risks that disasters pose and facilitate recovery after an incident. The EPA has worked extensively with states, utilities, and other community stakeholders in disaster preparedness, emergency response, recovery and rebuilding. Indicators can help communities identify environmental vulnerabilities, assets, and risks in the face of disasters. EPA and community stakeholders could use indicators to identify resilience priorities, design interventions, allocate funds, and measure progress.

## B. Methodology

Existing resilience indicators (Part C) were collected by a scientific literature search and put into an MS Access® database (Figure 1). Potential community environmental resilience indicators (Part D) were collected at two Community Environmental Resilience Index (CERI) workshops held at EPA in 2014. Indicators were proposed & discussed by 120 experts from EPA, ten other federal agencies, and non-federal scientific organizations.

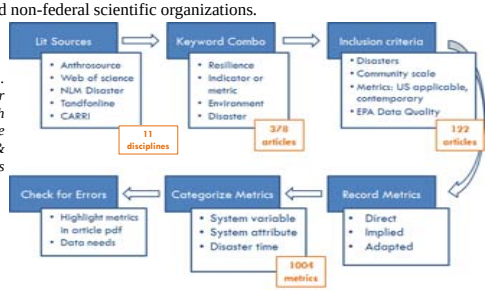


*Figure 1. Disaster literature search for resilience indicators & metrics*

Key terms: A *variable* is a factor that affects system resilience; *indicator* is a representation of trends and conditions in system variables; *metric* is a measurement of an indicator.

## C. What do existing resilience indicators tell us about community environmental resilience?

Existing resilience indicators and metrics from the disaster literature have limitations in their capacity to represent environmental and ecological trends and conditions that affect resilience (Figure 2). Only ten percent of indicators address environmental and ecological variables. The majority of indicators do not include metrics and data sources, impeding measurement of the indicator.

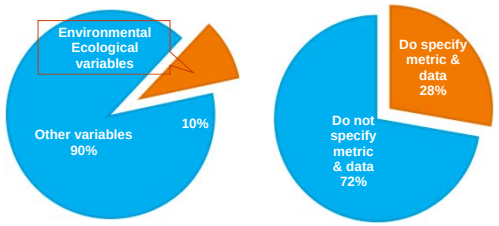


*Figure 2. Trends in disaster resilience indicators*

### What variables are being measured

Resilience indicators in the disaster literature provide information about different variables that affect community environmental resilience (Figure 3). Indicators of economic trends and conditions are most common, followed by infrastructure & built environment. The majority of demographic and environmental & ecological indicators are applicable to pre-disaster vulnerability and capacity. The majority of health & well-being and infrastructure & built environment indicators are applicable to post-disaster recovery.

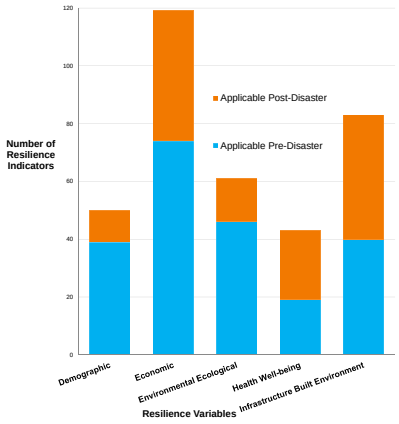


*Figure 2. Number of resilience indicators per variable category*

Key terms: *Environmental system* refers to socio-technical systems such as water and wastewater treatment plants that produce environmental services. *Ecological system* is a natural ecosystem such as a wetland or forest that provides ecosystem services.

## D. What are potential indicators of community environmental resilience?

Experts at the CERI workshops proposed & discussed potential community environmental resilience indicators. Many of these indicators were not found in the disaster literature and could fill in the gap in environmental indicators of resilience. I categorized indicators by variable. Table 2 shows potential indicators of community waste resilience. It includes indicators of pre-disaster preparedness and vulnerability, and post-disaster recovery. Socioeconomic and ecological variables affect community waste resilience, as well as infrastructure and technological considerations. After Hurricanes Sandy and Katrina, the presence of invasive species affected debris disposal options. After the 2014 chemical spill in the Elk River near Charleston, W. Va., over 20 million plastic water bottles were used to provide residents with drinking water. Two-thirds of area households had no curbside recycling, turning a water system problem into a waste management challenge.

*Table 2. Potential indicators of community waste resilience & variables measured*

| Community Waste Resilience Indicator | Variable |
|---|---|
| -Time to function: waste management<br>-Invasive species<br>-Percent green debris disposal | Environmental & ecological |
| -Transportation bottlenecks to disposal site<br>-Landfill capacity | Infrastructure & built environment |
| -Predesignation of debris sites<br>-Pretesting debris disposal technology | Disaster governance & planning |
| -Clean-up of key local places (park, school) | Sense of place & identity |
| -Environmental hazards per sq mi<br>-Contaminants in building stock | Health & well-being |
| -Race, class, ethnicity (in both disaster & disposal sites) | Demographic |
| -Contracts in place (recycling, waste haulers) | Economic |
| -Maturity of curbside recycling | Social networks & collective action |

## E. Next Steps

1. Access database of disaster resilience indicators: Add adapted environmental indicators that could measure resilience. Beta test database so EPA end users can find relevant indicators to design projects or track progress.
2. Community environmental resilience indicators: Find available metrics and data sources for potential indicators. Select indicators with input from EPA Program & Regional partners. Test indicators with community stakeholders. Use indicators to develop community self-assessment tools.

Acknowledgements: The CERI workshops were able to advance environmental resilience indicator development thanks to the AAAS Science & Technology Fellowship that supported my position, innovation funding from the National Homeland Security Research Center to host the workshops, the CERI team (Brendan Doyle, Susan Julius, Paul Lemieux, Regan Murray, Eli Walton, Cynthia Yund), and the input of workshop participants.

# Evaluation of Decontamination Methods Against *Bacillus atrophaeus* on Packaging Materials


**Kathryn Meyer** [1, 2], **Jenia Tufts** [1, 2], **and M. Worth Calfee** [2]

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National Homeland Security Research Center


## Abstract


Prior to transport of sample containment packages from the exclusion zone, effective decontamination procedures are necessary in order to prevent contamination of assets in the support zone and in support laboratories. Two sample package decontamination approaches (Clorox Healthcare™ Bleach Germicidal wipes and pH-amended bleach spray) were evaluated for decontamination efficacy on three packaging materials (corrugated fiberboard, polystyrene foam, and polyethylene). Liquid or aerosol preparations of *Bacillus atrophaeus* spores were deposited onto coupons, simulating two potential real-world modes of contamination. The inoculated surfaces were decontaminated with either pH-adjusted bleach liquid spray or a commercial sporicidal bleach wipe and allowed to dry overnight for 18-24 hours. Following decontamination, surfaces were sampled using a 3M sponge stick sampler to determine the abundance of viable spores remaining on the surface after treatment. To date, results suggest that decontamination efficacy was comparable between the two spore preparations for each of the coupon materials. Also, considering only the aerosol inoculated samples, polystyrene foam was more difficult to decontaminate than corrugated fiberboard or polyethylene. Additional work is ongoing to evaluate the current procedures for collection, packaging, and shipping of biological samples for their potential for cross-contamination. Results of these studies are intended to be used by on-scene coordinators to enhance sample collection, packaging, and decontamination protocols.

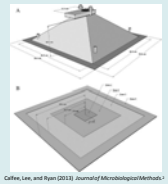

## Introduction

Shipment of biological specimens typically occurs through commercial mail and/or package couriers. During a biological incident, samples are collected and then transported out of the exclusion zone through a decontamination line, which separates the exclusion and support zones. Decontamination procedures are rendered on sample packaging materials, and are meant to reduce the risk of contaminants being transported into the support zone, where samples are further packaged and shipped to supporting laboratories. Although the current CDC surface sampling procedure for *B. anthracis* spores from smooth, non-porous surfaces[1] requires both the primary and secondary containment sample bags to be decontaminated with pH-amended bleach, the procedure does not suggest decontamination of the actual shipping containers that will transport the specimens to their support laboratory. Cross-contamination of these shipping containers/packages may pose a potential threat not only to the couriers, but also to the support laboratories who receive these packages alongside their everyday shipments. Therefore, the present study was designed to evaluate the effectiveness of sample packaging decontamination procedures using a *B. anthracis* surrogate, *B. atrophaeus*, and several sample transport shipping materials.

## Test Design

**Preparation**

- Liquid spores
- Aerosolized spores



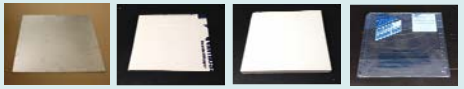
Calfee, Lee, and Ryan (2013) *Journal of Microbiological Methods*[2]

**Decontaminants**

- Clorox™ Germicidal wipes
- pH-amended Bleach spray

**Coupon Materials**

- Stainless Steel
- Cardboard
- Styrofoam
- Low-Density Polyethylene



## Test Methods

### Inoculation

- Liquid Deposition
  - Coupons were inoculated with 10 × 100 µl drops of 2 x 10 $^{6}$CFU of *B. atrophaeus* spores in an hourglass pattern.

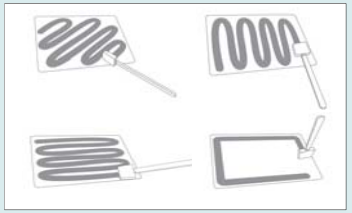
- Aerosol Deposition
  - Coupons were inoculated with 2 x $10^{7}$ aerosolized *B. atrophaeus* spores using a metered dose inhaler through an aerosol deposition apparatus[2] and placed at room temperature for 18-24 hours for spore deposition to occur.

### Decontamination

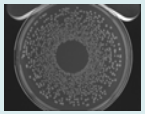
- Clorox Germicidal Wipes
  - Towelette was folded 2 times. Surface of coupon was wiped in 3 directions, each time the towelette was folded inward before use. Coupons air dried for 18-24 hours.

### Sample Collection

- Sponge-stick samplers were used to collect samples from the coupon surfaces after drying for 18-24 hours. Surfaces were sampled in 4 directions and sponges were extracted in PBST in a stomacher.

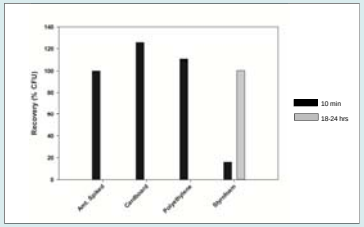


- Undiluted and serially diluted sample extracts were plated onto tryptic soy agar (TSA) and CFU were enumerated to determine survivorship (viable spore abundance).

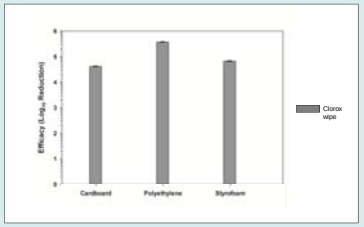


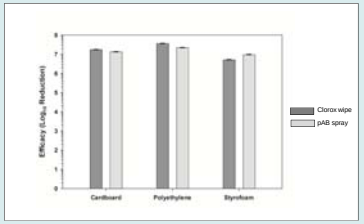
## Results

### Decontaminant drying time determination




### Liquid-inoculated coupons




## Summary of Results

- The Clorox wipe showed higher decontamination efficacy than the pAB spray, potentially due to the physical removal associated with wiping.
- Liquid-inoculated coupons (known spots of inoculation) were more easily decontaminated than aerosol-inoculated coupons (uniform spatial distribution).
- Aerosol-inoculated styrofoam coupons were more difficult to decontaminate than aerosol-inoculated cardboard or polyethylene.



## References


1. CDC (2012) Surface sampling procedures for *Bacillus anthracis* spores from smooth, non-porous surfaces. Atlanta, GA: Centers for Disease Control and Prevention.
2. Calfee MW, Lee SD, and Ryan SP. (2013). "A Rapid and Repeatable Method to Deposit Bioaerosols on Material Surfaces." Journal of Microbiological Methods **92(3)**: 375-380.

# Micro-vapor Chambers and Design of Experiments Approach for Investigating Vaporous Decontaminants

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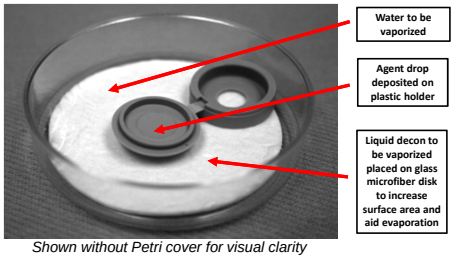
[1]US Army Edgewood Chemical Biological Center, APG, MD; [2]Leidos, Abingdon, MD

## Introduction

Vaporous decontamination chemistries are ideally suited to homeland response scenarios. They provide decontamination for all exposed surfaces, do not create runoff or transfer contamination, and can greatly reduce manpower requirements. Furthermore, they may reduce hazards associated with applying solution-based decontaminants. However, efficient investigation of these chemistries is greatly hampered when using standard vapor test chambers as typically only one condition can be assessed per test session per test chamber due to the long exposures required and the time associated with bringing the concentration to equilibrium in a large chamber. A highly efficient approach for investigating the vaporous decontamination of chemical agent contaminated surfaces was recently explored using a combination of micro-vapor chambers and a design of experiments (DOE) approach. The statistical DOE approach coupled with the micro-vapor chambers was used to identify the most influential decontamination process factors associated with using hydrogen peroxide, formic acid and acetic acid vapors as decontaminants.

## Micro-vapor Chambers

- 2 in diameter Petri dishes served as micro-vapor chambers
- Agent placed on plastic holder ensured only vaporous exposure
- Vapors were generated by adding calculated volumes of liquid decontaminant and water (based on interval volume of Petri) to Petri dishes immediately before placing in temperature controlled enclosure at 40 or 50 °C
- Glass microfiber disk in bottom of Petri increased surface area of added liquids to aid in volatilization
- Plastic holder was extracted following vaporous exposure using 2-propanol and analyzed via LC-MS to assess remaining agent
- Numerous micro-chamber tests were conducted per test session permitting examination of multiple vaporous decontaminants, concentrations and conditions using hydrogen peroxide and acidic vapors.
- The use of multiple micro-chamber tests per test session provided much greater throughput and efficiency than that provided by standard vapor exposure chambers




*Shown without Petri cover for visual clarity*

## DOE Process Factors

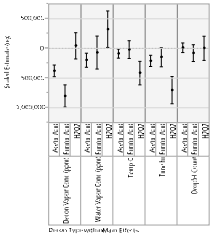
A DOE study was devised to evaluate 3 decontaminant types (acetic acid, formic acid, hydrogen peroxide) with a total of 6 process factors (5 continuous, 1 categorical). Estimation of main effects, second order effects and all 2-way interactions was provided by the design. The DOE design was created using a D-Optimal criteria within JMP 11 statistical software (SAS Institute Inc., Cary, North Carolina)

| Factor | | Factor Level | | |
|---|---|---|---|---|
| | | Low | Medium | High |
| Decon Vapor Level | Acetic Acid (calc ppm) | 1,000 | 4,000 | 10,000 |
| | Formic Acid (calc ppm) | 100 | 300 | 1,000 |
| | $H_2O_2$ (calc ppm) | 1,500 | 3,500 | 5,500 |
| Water Vapor Level (calc ppm) | | 400 | 1,200 | 4,000 |
| Temperature (°C) | | 40 | ------- | 50 |
| Time (h) | | 2 | 3 | 4 |
| Drop Count | | 1 | ------- | 2 |

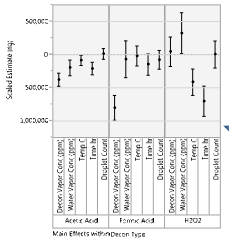
## Main Effects Scaled Estimates

The DOE was analyzed by fitting scaled estimates for the process factors to permit direct comparison of effect influences within a decontaminant or between decontaminants.

**Decontaminant Type within Main Effects**



**Main Effects within Decontaminant Type**



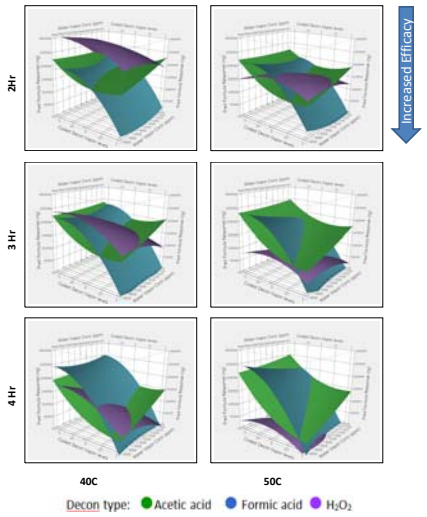

**Summary of Main Effects by Decontaminant Type (scaled effect estimates (ng))**

| Decon Type | Decon Vapor | Water Vapor | Time | Temp | Drop# |
|---|---|---|---|---|---|
| Acetic Acid | -374,288 | -194,855 | -206,436 | -83,470 | 15,355 |
| Formic Acid | -797,789 | -65,249 | -145,502 | -15,925 | -74,676 |
| $H_2O_2$ | 48,083 | 328,507 | -698,909 | -411,833 | 4,951 |

| |
|---|
| Improves Efficacy |
| No Effect |
| Reduces Efficacy |

## Response Surface Grids

The relationships between the process factors and the response variable are easily visualized for each decontaminant type with increasing exposure period and by temperature.




**Acetic acid** - the important main effects to increase decontamination performance were decontaminant vapor, and to a lesser degree water vapor and exposure time

**Formic acid** - the only important main effect to increase decontamination performance was the decontaminant vapor

**$H_2O_2$** - the main effects to increase efficacy were time, and temperature, while water vapor was found to reduce efficacy. This negative effect of water vapor on hydrogen peroxide efficacy is attributed to its ability to reduce peroxide vaporization. Since the hydrogen peroxide vapor source is an aqueous solution containing 41% water (59% Vaprox solution) additional water may reduce the vapor pressure due to the similarity of the two compounds.

## Prediction Model Based on Optimal Settings

The DOE results were used to fit a predictive model which included all main, 2-way interactions and 2nd order effects. The predictive model was then used to find the factor settings for optimal efficacy. The optimal factor settings based on the predictive model were tabulated. As would be expected, the optimal settings included the highest temperature (50 °C) and longest exposure time (4 h), in addition to decon vapor levels at or near the high level, with the exception of hydrogen peroxide which was predicted to provide optimal performance at the low peroxide level (1,500 ppm). Additionally, while hydrogen peroxide and formic acid efficacies were predicted at 2-6 and 1-2 log reductions of contaminant respectively, acetic acid was estimated to provide only 82 % neutralization efficacy.

**Predicted Optimal Factor Settings**

| Decon | Efficacy | Decon Vapor (ppm est) | Decon Vapor level | Water Vapor (ppm est) | Water Vapor level | Temp (°C) | Time (h) | Drops# |
|---|---|---|---|---|---|---|---|---|
| Acetic Acid | 82% | 9,400 | Near High | 4,000 | High | 50 | 4 | 1 |
| Formic Acid | 1-2 log reduction | 1,000 | High | 400 | Low | 50 | 4* | 2 |
| $H_2O_2$ | 2-6 log reduction | 1,500 | Low | 400 | Low | 50 | 4** | 2 |

* Model estimates 2.5 hr to achieve 100% efficacy.
** Model estimates 2.3 hr to achieve 100% efficacy.

## Conclusions

- The combination of micro-vapor chambers and design of experiments (DOE) provided a highly efficient approach for investigating the vaporous decontamination of chemical agent contaminated surfaces
- The approach permitted the rapid evaluation of 3 decontaminant types (hydrogen peroxide, acetic acid, and formic acid) with a total of 6 process factors (decontaminant type, decontaminant vapor level, temperature, time, agent drop size, and humidity)
- Estimation of main effects, second order effects and all 2-way interactions was also provided by the design
- The predictive model created via the DOE allowed for the estimation of optimal efficacy settings using all main, 2-way interactions and 2nd order effects

## Acknowledgements

- Iain McVey (STERIS Corp.) for financial and technical support
- Michael Chesebrough (OptiMetrics, Inc.) for analytical support
- Michelle Sheahy and Stefanie Quinones (Leidos Corp.) for laboratory operational assistance
- Jennifer Weitzel and Nicholas Sapienza (Leidos Corp.) for technical support

# Should I coat my building?
# Protecting buildings from CBR contamination

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Buildings can become contaminated in a CBRN attack, with contamination penetrating into the walls. Radionuclides can readily absorb into a substrate in a few weeks [i,ii], and be driven deeper by weathering or water based wet decontamination. As a result, contaminated buildings could need to be subjected to more aggressive decontamination techniques or even destroyed.

Specialist impermeable coatings are used to prevent contamination permeating into porous indoor surfaces. In industry (e.g. nuclear) and medicine (e.g. medical laboratories) the use of coatings improves the ability to undertake in-situ routine and end-of life decontamination. Could a similar approach mitigate CBRN contamination?

Should we apply Commercial Off The Shelf (COTS) coatings to our houses, office blocks and heritage monuments to enable a cheaper recovery phase and minimise waste? Could the benefits extend to neutralising chemical contamination and killing biological contamination?

This project looked at the possibility of using commercial and novel coating technologies as protection against building contamination. Information from open sources was used to compare the marketing claims against building requirements and the technologies for their ability to repel or "deal with" chemical (C), biological (B), or radiological (R) contaminants.

## Building conservation issues

Building conservation is a conservative sphere: the application of protective treatments is avoided due to their possible adverse effects. These effects can be detrimental to the building integrity compared to existing cleaning regimes.

Damage can be caused by preventing or slowing down the normal water movement out of the surface: When water movement is unhindered, salts within the stone are carried to the surface where they may be unsightly but can be washed off. But trapped water deposits the salts behind the surface layer, where thermal and crystallisation stresses can eventually cause the stone to spall, leaving a weaker surface that is more vulnerable to natural weathering. Hence, impermeable treatments, even 'breathable' ones, should only be applied to parts of buildings that are otherwise water-tight. In addition, natural stones vary in properties (e.g. vapour and liquid transport coefficients) across and within stone types and so performance and criteria must be judged on a case by case basis.


Photo: NPS files

Figure 1: Continuous spalling and erosion result in severe stone decay


Photo: NPS files

Figure 3: Salt efflorescence appears as a fine, white, powdery substance on a uncoated brick wall and but is only unsightly and can be washed off.

## Use of COTS treatments

With technological advances, coatings claim to offer greater repellency and self-cleaning properties. In addition there is evidence of increasing use on buildings of significance (See Figures 7 and 8). A selection of COTS treatments was assessed against a list of desirable criteria to evaluate whether their deployment would provide tangible benefits for CBR protection and be compatible with building conservation.

Criteria Order of Importance

**A treatment should:**

- Repel water, oils and particulates
- Be invisible
- Be water vapour permeable
- Be inert towards the substrate but functional against the contaminant
- Not require aggressive pre-treatments
- Be reversible
- Be non-prejudicial to later interventions
- Be long-lived

## Products fall in 3 main technology groups

### Silanes/siloxanes blends used with Sol-Gel process (Figure 3)

**Pros:**

- Simple control of functionality gives proven hydrophobicity over time (alkyl groups), or oleophobicity (polar groups), or both
- On impregnation, mostly invisible and UV resistant
- Vapour permeable

**Cons:**

- Vulnerable to acid based contaminants
- Ineffective against Chemical Warfare (CW) agents even when functionalised
- Possible staining with higher loadings
- Variable durability
- Not reversible, may prejudice other treatments

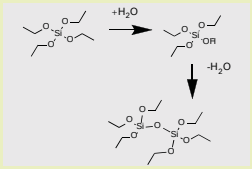


Figure 3: Tetraethyl orthosilicate (TEOS) is the basis for the Sol-Gel process. Hydrolysis, followed by precipitation, results in the formation in-situ of a silica polymer

Figure 4: Silica's reactive OH- groups can be functionalised with alkyl groups to impart hydrophobicity

### Fluorocarbon suspensions

**Pros:**

- Omniphobic
- Invisible
- Water vapour permeable, inert towards matrix
- UV resistant

**Cons:**

- Vulnerable to abrasion
- Perfluorooctanoic acid (PFOA) ban in US and likely in EU, yet if <C6 may not repel CW effectively
- Prejudicial to later interventions

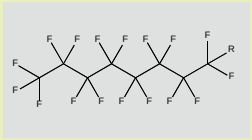

Figure 5: Repellency depends on the length of the fluorocarbon chain

### Nano-titania additions

**Pros:**

- Super-hydrophylic behaviour: water washes break-down products and particulate contamination away
- Photocatalysis results in biocidal properties, and organic pollutants (CW) are neutralised/oxidised
- Evidence of emerging in-situ use and testing on historic buildings [iii]
- Invisible, water vapour breathable

**Cons:**

- Not inert towards substrate
- Only one COTS product found for in-situ outdoor use
- Longevity not proven

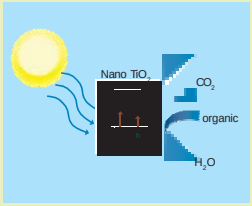


Figure 6: Photocatalysis diagram

### Emerging technologies:

**Experimental treatments bring improved and multiple benefits and reduced limitations:**
Functionalised silanes/siloxane blends for increased penetration and water repellence coupled with:

- Nano-titania for photo-catalysis [iv]
- Other nano- additions for extra functionality e.g. silica as a consolidant, e.g. silver as a biocide [v,vi,vii]

**Polysacharride coatings with:**

- Fluorinated polylactic acid as a biodegradable water and oil repellent with biocidal properties
- Boric acid or $AgNO_3$ for biocidal properties plus Sol-Gel [ix]

## Product assessment

14 COTS products were identified from marketing claims spanning 3 main technology types. They claim to be invisible AND breathable AND offer some repellency or means of self-cleaning. However, none claimed to meet all the other criteria: those least satisfactorily met or documented were breathability rate, reversibility and longevity.

| Technology | Number of products | Repellency: Oil | Repellency: Water | Repellency: Particles | Invisible | Water vapour breathable | Inert towards substrate | Durability (years) | Added benefits: Self or Easy Cleaning | Added benefits: Biocidal | Future Proof: Allow for re-treatment | Future Proof: Reversible |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stone | 5 | (✓) | ✓ | ✓ | (✓) | ✓ | | (0.5-8) | ? | | | |
| Silicone | 2 | (✓) | ✓ | ✓ | (✓) | ✓ | | | ? | | | |
| Blends of these | 2 | (✓) | ✓ | ✓ | ✓ | ✓ | | (15 25) | EC | | | |
| Fluoroemulsions | 5 | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | |
| $TiO_2$ | 1 | ? | | | ✓ | | | ✓ | ✓ | ✓ | | |
| Silane + $TiO_2$ | 1 | (✓) | ✓ | ✓ | ✓ | ✓ | | 5-10 | SC | ✓ | | |

Table 1: Summary of products claims by technology type against selection criteria. (Brackets indicate that functionality varies depending on formulation or application, blanks mean that no claims were made)

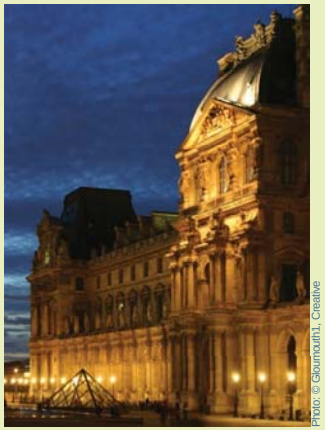
Photo: © Gbaumouth1, Creative Commons

Figure 7: The Louvre walls in Paris, treated with a silane based coating.


Photo: © Ad Meskens / Wikimedia Commons

Figure 8: The Bruges City Hall (Belgium), coated with a silane/siloxane blend.

## Conclusions:

- Protection is possible against water, oils and particulate contamination
- No universal treatment, but evidence of world-wide use
- Current technologies all have drawbacks and limited performance data against standards (not real world)
- Emerging formulations could harness advantages of multiple technologies and minimise drawbacks. When commercialised, these should be tested against CBR contamination

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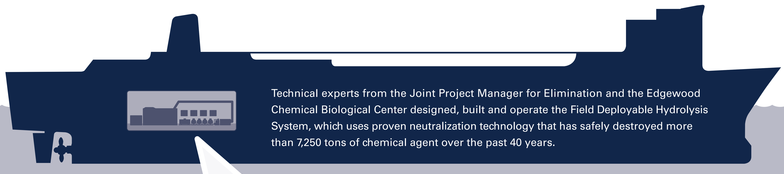
i Maslova (2013) Journal of Environmental Radioactivity, (125) 74-80
ii Lee (2013). EPA/600/R-12/068
iii.Pinho and Mosquera (2013) Applied Catalysis B: Environmental 134–135, 205– 221
iv.Pinho ( 2013) Applied Surface Science, (275) 389-396 v Zaraga (2009), Journal of Cultural Heritage, (11-2), 138–144
vi Ferri (2011), Journal of Cultural heritage, (12-4), 356-363.
vii Kapridaki (2013), Progress in Organic Coatings, (76), 400-410
viii. Sacchi (2012), 12th International Congress on the Deterioration and Conservation of Stone, 248-257
ix. Eyssautier-Chuine (2012), 12th International Congress on the Deterioration and conservation of Stone (136-144)

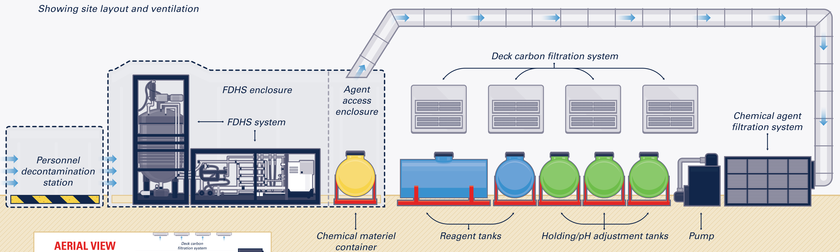
# CAPE RAY FIELD DEPLOYABLE HYDROLYSIS SYSTEM OPERATIONS

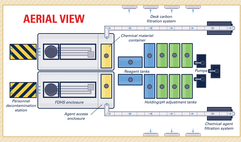


Technical experts from the Joint Project Manager for Elimination and the Edgewood Chemical Biological Center designed, built and operate the Field Deployable Hydrolysis System, which uses proven neutralization technology that has safely destroyed more than 7,250 tons of chemical agent over the past 40 years.

## SIDE VIEW

*Showing site layout and ventilation*

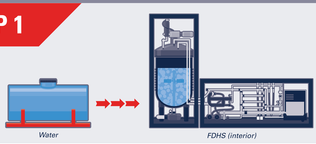



Each side of the enclosure is ventilated to carbon filters to remove contaminants. Air from the operational deck of the ship is passed through additional carbon filtration prior to exiting the ship.

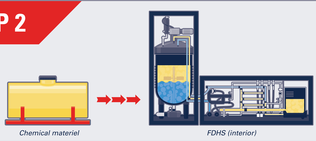
## DESTROYING **HD**

### STEP 1




Hot water is fed into the mixing vessel.

### STEP 2




Mustard agent is fed into the mixing vessel and mixed with the hot water.

### STEP 3


The HD molecule reacts with two molecules of water.

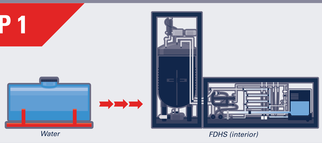
Two chlorine atoms are removed from the HD, forming a new, less toxic compound.

### STEP 4


The effluent is pH adjusted with sodium hydroxide. The end solution is a mixture of water, thiodiglycol and sodium chloride.

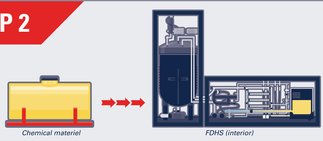
## DESTROYING **DF**

### STEP 1




Room-temperature water is added to the static mixer manifold.

### STEP 2




DF is added to the static mixer manifold and mixed directly with the water.

### STEP 3


The DF molecule reacts with two molecules of water.

Two fluorine atoms are removed from the DF, forming a new, less toxic compound.

### STEP 4


The effluent is pH adjusted with sodium hydroxide. The end solution is a mixture of water, methylphosphonate and sodium fluoride.

### STEP 5


Operators transfer effluent from the interim holding tanks to isotainers for storage. Effluent is delivered to an OPCW-selected commercial treatment facility.

# CRP OVERVIEW

The Critical Reagents Program (CRP) serves as the principal resource of high quality, validated, and standardized biological reference materials, reagents, and assays that meet the technology-development and sustainment needs of the Department of Defense (DoD) and its partners. In 2007, the CRP instituted program-wide quality initiatives to integrate and execute formal quality management systems into all aspects of its program operations. The CRP Product Support Office is ISO: 9001-2008 certified; CRP production activities and associated reference standards qualification are registered under ISO Guide 34 and relevant ISO 17025, where appropriate. As such, the CRP provides the highest quality biological detection solutions to the DoD, international allies, and homeland defenders including the Department of Homeland Security (DHS) BioWatch Program.

# CRP SUPPORT OF PARTNERS

The CRP supplies antigens (inactivated organisms), genomic material, antibodies, PCR detection assays, electrochemiluminescence (ECL) immunoassays, and lateral flow immunoassays (LFIs). The program also provides technical support to various programs within the US Government and the DoD Chemical and Biological Defense Program.

## LEGEND

- REF MATERIALS
- ASSAYS
- SERVICES
- RESOURCES

# CRITICAL REAGENTS PROGRAM

*Products & Services*

## REAGENTS

Biological reference materials

- **UNIFIED CULTURE COLLECTION (UCC)** *(microbial strains)*
- **ANTIGENS** *(inactivated)*
- **GENOMIC MATERIAL** *(RNA & DNA)*
- **ANTIBODIES**

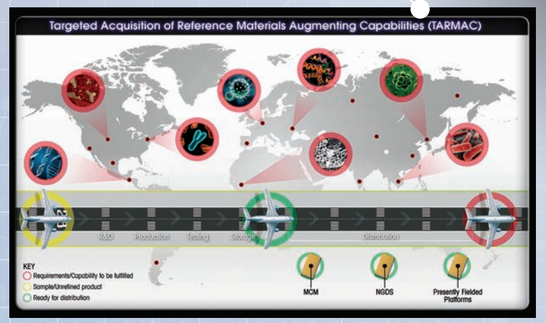
## COLLECTION LABS

Biological reference materials and reagents

- **UCC** *(microbial strains)*
- **EDGEWOOD CHEM BIO CENTER (ECBC)** *(antibodies & genomics)*
- **WEST DESERT TEST CENTER @ DUGWAY PROVING GROUND (DPG)** *(antigens)*

## TARMAC

Targeted Acquisition of Reference Materials Augmenting Capabilities




## ASSAYS

Biological detection solutions

- **LATERAL FLOW IMMUNOASSAYS (LFI)**
- **ELECTROCHEMILUMINESCENCE (ECL) ASSAYS**
- **POLYMERASE CHAIN REACTION (PCR) ASSAYS**

## OSCAR

Ordering System for CRP Assays and Reagents

- **ANTIGENS** *(inactivated)*
- **GENOMIC MATERIAL** *(RNA & DNA)*
- **ANTIBODIES**
- **LFI ASSAYS**
- **ECL ASSAYS**
- **PCR ASSAYS**

## CRPµTIC

CRP (microbial) Threat Information Center

- **PRODUCT DATA**
- **GENOTYPIC DATA**
- **PHENOTYPIC DATA**
- **STRAIN META DATA**
- **SAMPLE DATA**

# RECENT DEVELOPMENTS

In 2012, CRP launched their TARMAC initiative. TARMAC stands for the Targeted Acquisition of Reference Materials Augmenting Capabilities, and works to ensure that emerging threats and capability gaps are effectively addressed by ensuring new pathogen collections are relevant to the current mission space. Strains acquired through TARMAC are used to evaluate and improve the performance of existing assays and expand the products that are offered to CRP customers.

As a complement to TARMAC, the CRP created a pathogen data resource called CRPµTIC (the CRP (microbial) Threat Information Center). This data resource contains strain metadata, and phenotypic and genotypic characterization data on the strains contained in the Unified Culture Collection (UCC). The UCC serves as the foundation for a wide variety of CRP products, so this data greatly enhances the information available to a wide variety of CRP stakeholders. All strain acquisitions through the TARMAC initiative are also accessioned into the UCC and characterized for inclusion in CRPµTIC.

In FY15, the CRP introduced online ordering for its customers. The system, dubbed OSCAR (Ordering System for CRP Assays and Reagents), integrates customer ordering with the entire fulfillment process — it's truly an end-to-end solution that allows customers to place orders online and have 24-hour visibility into their order status and history. The CRP office, government support labs, and contracted storage and distribution partners will all be using the system, so orders will always be updated in real time.

# CONTACT US

The CRP protects the warfighter and the nation by working with top scientific experts from the DoD & other biodefense partners to provide a comprehensive portfolio of world-class materials, reagents, assays, and biological detection technologies available.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The CRP logo is a gargoyle, signifying protection and guardianship.