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Challenges in the Decontamination of Chemical and Biological Warfare Agents and Other Biological Pathogens

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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

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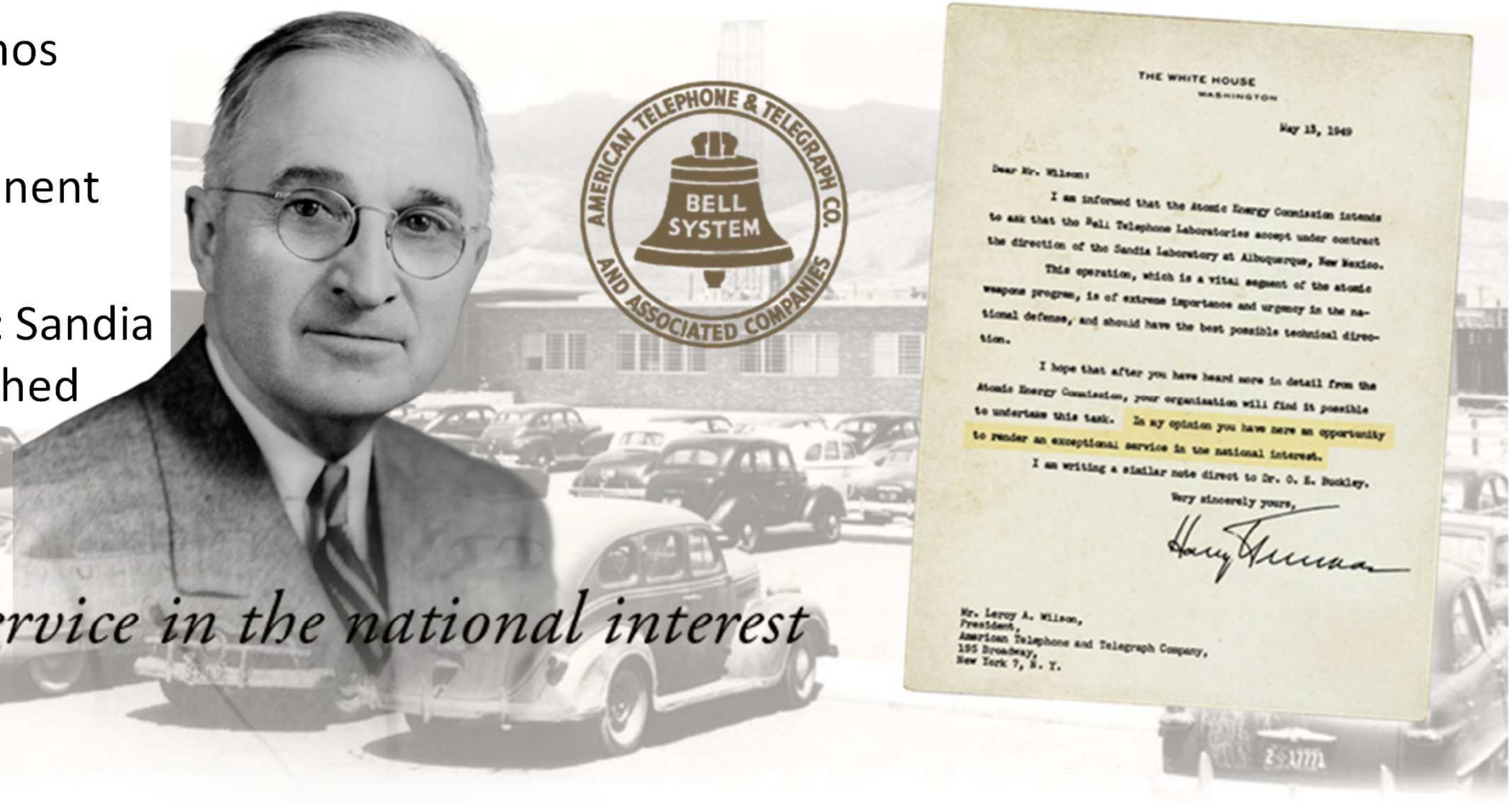
Sandia History

- July 1945: Los Alamos creates Z Division
- Nonnuclear component engineering
- November 1, 1949: Sandia Laboratory established



THE WHITE HOUSE
WASHINGTON

May 13, 1949

Dear Mr. Wilson:

I am informed that the Atomic Energy Commission intends to ask that the Bell Telephone Laboratories accept under contract the direction of the Sandia Laboratory at Albuquerque, New Mexico.

This operation, which is a vital segment of the atomic weapons program, is of extreme importance and urgency in the national defense, and should have the best possible technical direction.

I hope that after you have heard more in detail from the Atomic Energy Commission, your organization will find it possible to undertake this task. In my opinion you have here an opportunity to render an exceptional service in the national interest.

I am writing a similar note direct to Dr. O. E. Buckley.

Very sincerely yours,

Harry Truman

Mr. Leroy A. Wilson,
President,
American Telephone and Telegraph Company,
195 Broadway,
New York 7, N. Y.

Exceptional service in the national interest

Sandia National Laboratories

Sandia Sites

Albuquerque, New Mexico

Livermore, California

Kauai, Hawaii

Waste Isolation Pilot Plant, Carlsbad, New Mexico

Pantex Plant, Amarillo, Texas

Tonopah, Nevada

Sandia National Laboratories

Overview of Sandia National Laboratories

Federally Funded Research and Development Center

We develop technologies for <u>national security applications</u> to:

- Sustain, modernize and protect our nuclear arsenal
- Prevent the spread of weapons of mass destruction
- Provide new capabilities to our armed forces
- Protect our national infrastructures
- Ensure the stability of our nation's energy and water supplies.
- Defend our nation against terrorist threats

Includes CBRN Threats

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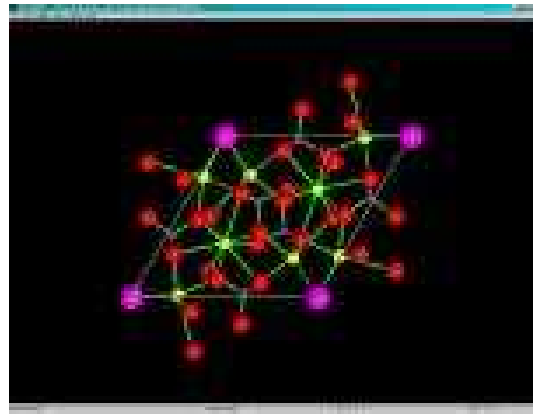
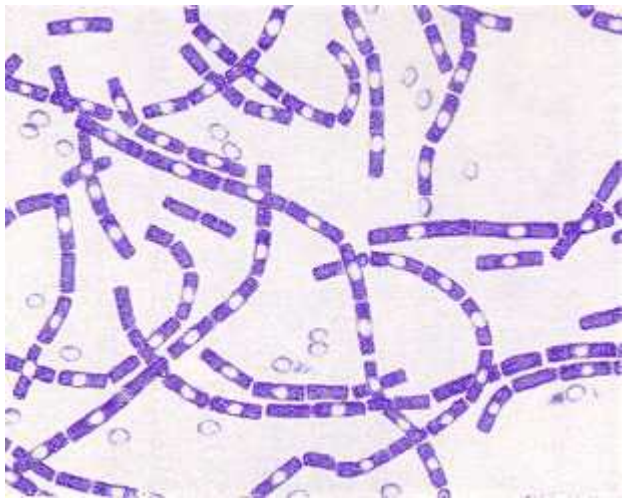
A primary mission at Sandia is to address the CBRN threat

Chemical





Biological

Chemical Warfare Agents (e.g., nerve agents, mustard gas)

Biological Warfare Agents (e.g., anthrax)

Nuclear

Radiological

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CBW agents are extremely toxic and have been used both by terrorists and in military operations

British soldiers blinded by a Mustard attack in World War I

The 1995 Sarin release in the Tokyo subway killed 12 people

The October 2001 anthrax releases in office buildings and postal facilities in the US killed 5 people

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Biological Warfare Agents are divided into four categories

Spore-forming Bacteria

Bacillus anthracis spores

- **Persistent**
- **Highly Transportable**
- **High Resistance to Decon**
- **Non-contagious**

Vegetative Bacteria

Yersinia pestis

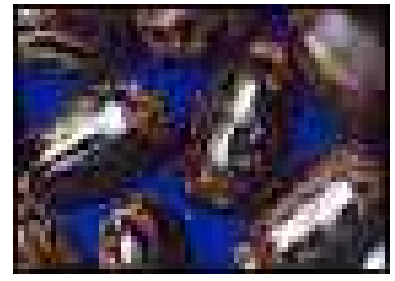
- **Non-Persistent**
- **Transportable (Mod)**
- **Moderate Resistance to Decon**
- **Contagious (Low)**

Viruses

Variola major

- **Non-Persistent**
- **Transportable (Low)**
- **Low Resistance to Decon**
- **Contagious (High)**

Biotoxins



Ricin

- **Moderate Persistence**
- **Transportable (Mod)**
- **Moderate Resistance to Decon**
- **Non-Contagious**

Images from: http://emergency.cdc.gov

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Chemical Warfare Agents are categorized by their health effect

Blistering Agents (Skin & Tissue)

- Persistent
- Transportable
- Moderate Toxicity
- Low Volatility Liquids

Mustard (HD)

Lewisite (L)

Nerve Agents (Nervous System)

- Persistence Varies
- Transportable
- Moderate Toxicity
- Volatile Liquids

Sarin (GB)

VX

Choking Agents (Lungs)

- Low Persistence
- Transportable
- Low Toxicity
- Gas

Chlorine (Cl)

Phosgene (CG)

Blood Agents (Oxygen Transport)

- Low Persistence
- Transportable
- Low Toxicity
- Volatile Liquids or Gas

Hydrogen Cyanide (AC)

Cyanogen Chloride (CK)

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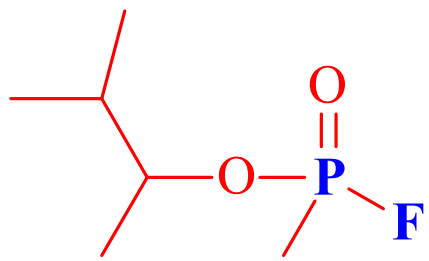
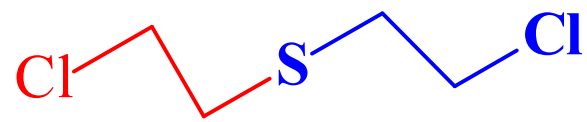
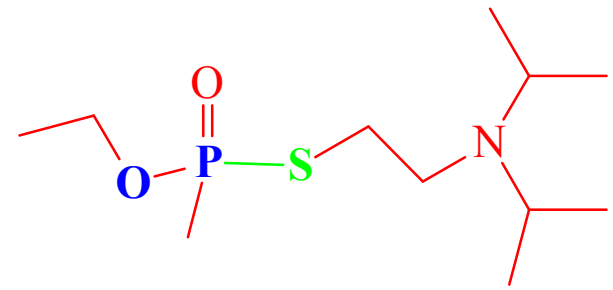
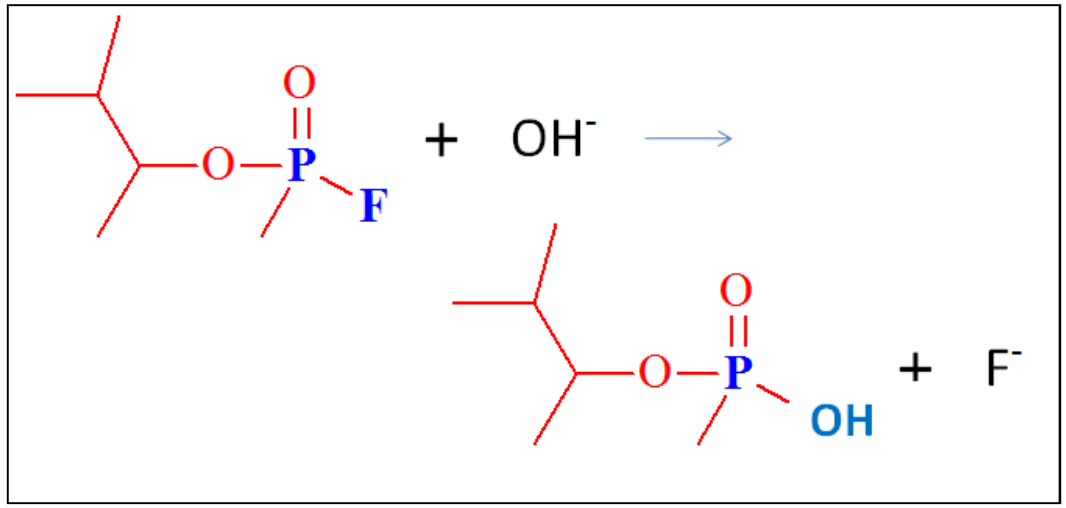
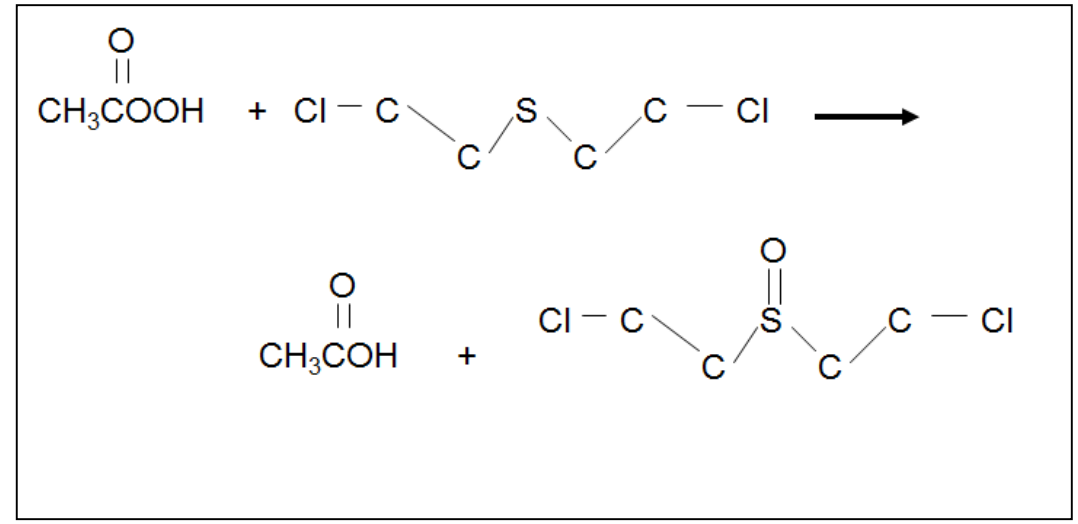
The molecular structure Chemical Warfare Agents are quite different

	Soman (GD)	Mustard (HD)	VX
Chemical Agents			



Hydrolysis



Oxidation

The differences in chemical structure can make decontamination of chemical warfare agents very challenging

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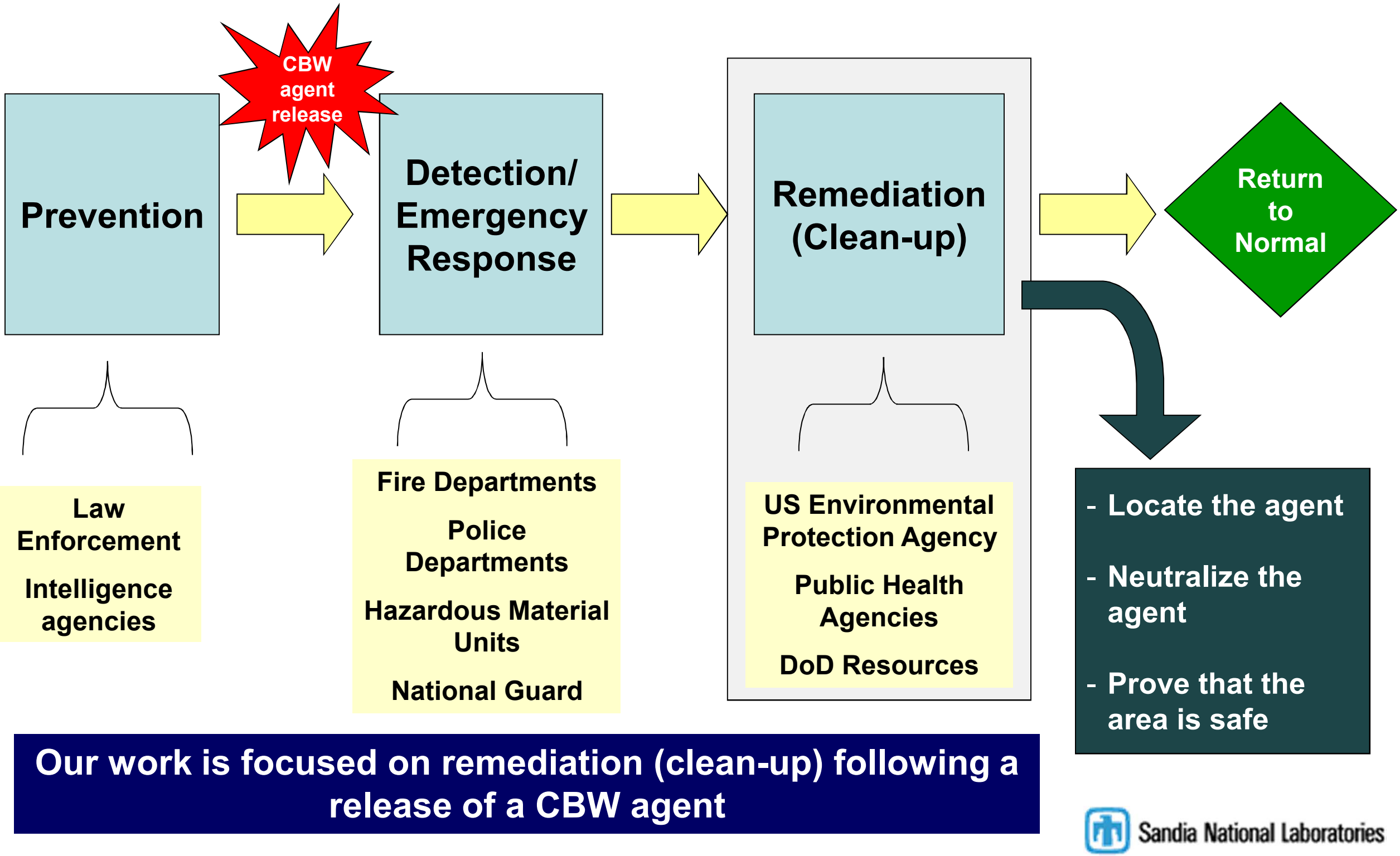
Work at Sandia is focused on prevention, response, and recovery



Our work is focused on remediation (clean-up) following a release of a CBW agent

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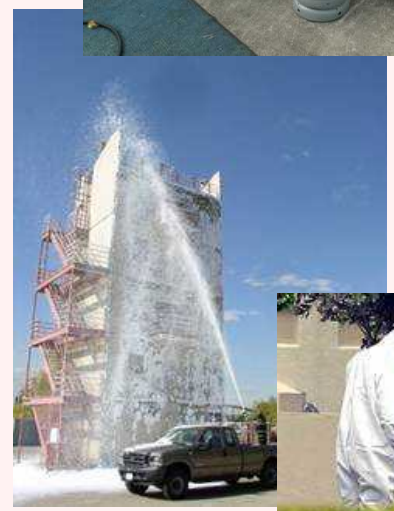
The remediation/recovery process can be very complex

Phases of Remediation	Characterization	Decontamination	Clearance

Each phase represents difficult challenges:

- Many types of materials
- Sensitive equipment that cannot be damaged
- Hidden surfaces which may require liquid and gas decon technologies
- Large number of samples
- Working in PPE
- Limited resources
- Limited waste disposal options
- Pressure to re-open critical facilities quickly



A primary objective is make each of these phases better and faster

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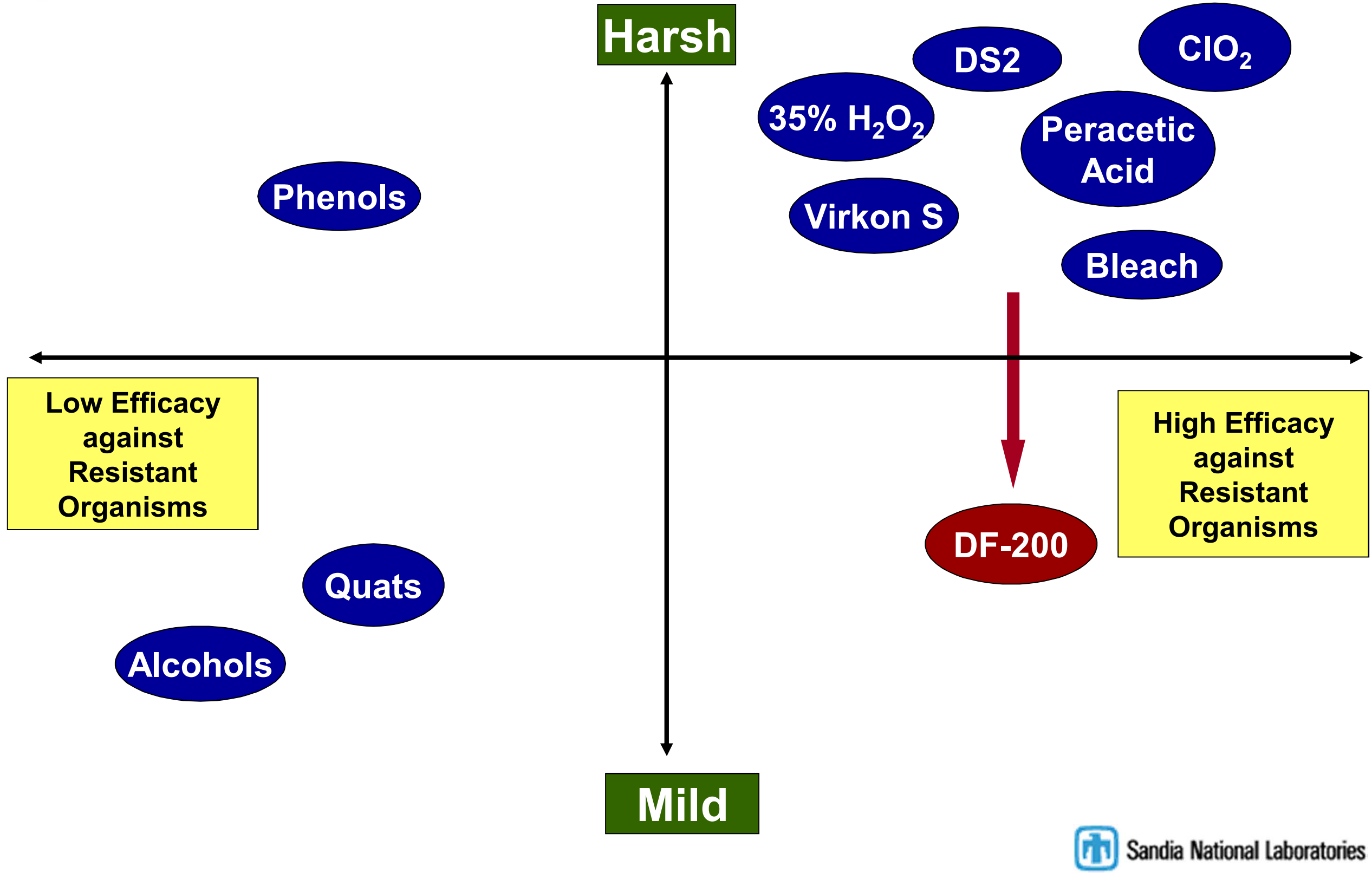
Our challenge was to develop a less toxic and corrosive decontaminant for counter-terrorism and military applications



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Sandia Decon Formulation (DF-200)

How Does it Work?

Components

Foam Component (Surfactants, mild solvents, buffers)

Peroxide (7.9% Solution)

Novel Activator

Mix

Formulation

Synergistic formulation (multiple reactive species)

Spray, Foam, Mist, or Gel

Multiple Uses

Kill of BW Agents

Kill of Bio Pathogens

Neutralization of CW Agents

Neutralization of TICs

Final peroxide concentration is ~3.6%

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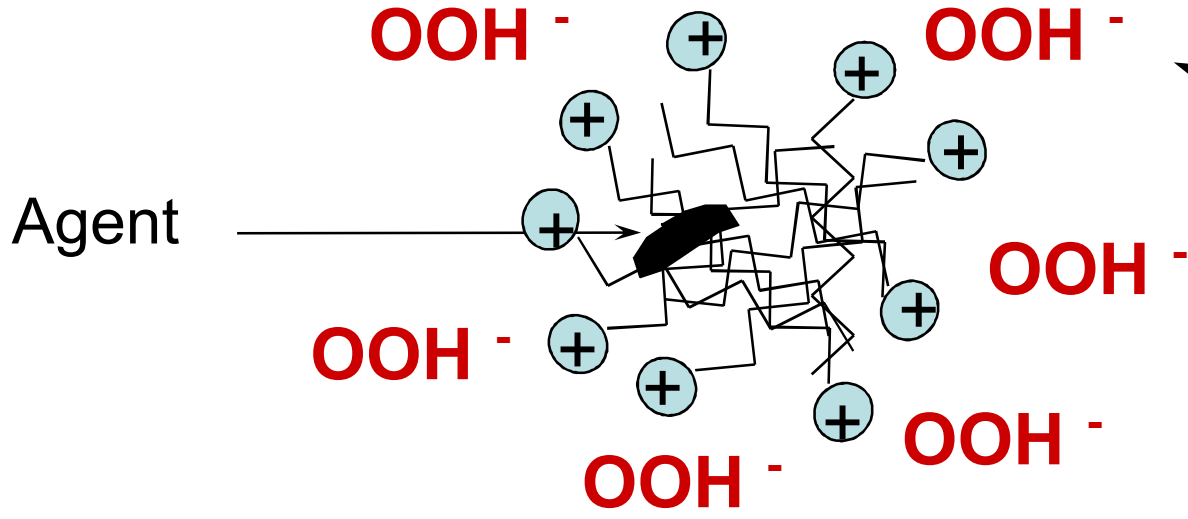
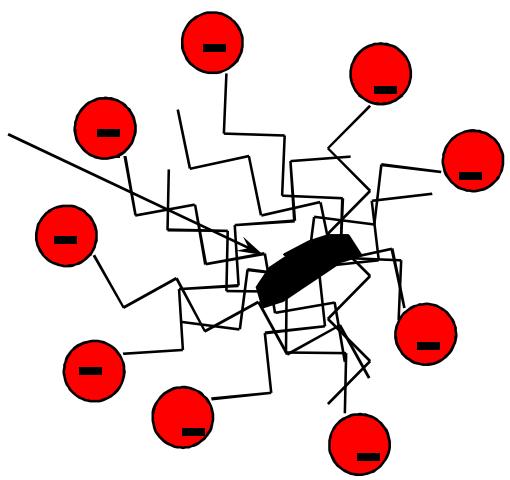
Decontamination Using Micellar Catalysis



Cationic micelle, catalysis

Agent



Anionic micelle, no catalysis

OOH -

OOH - OOH -

OOH - OOH - OOH -

OOH -

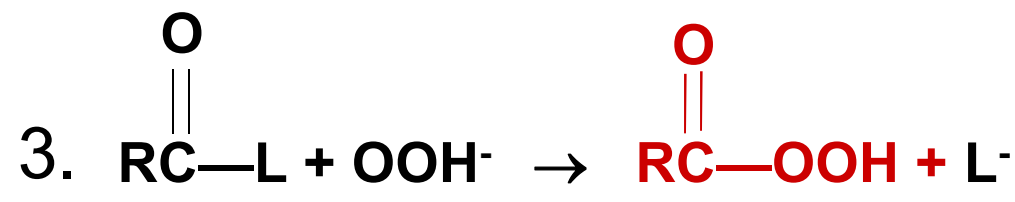
Catalyst repelled
from micelle

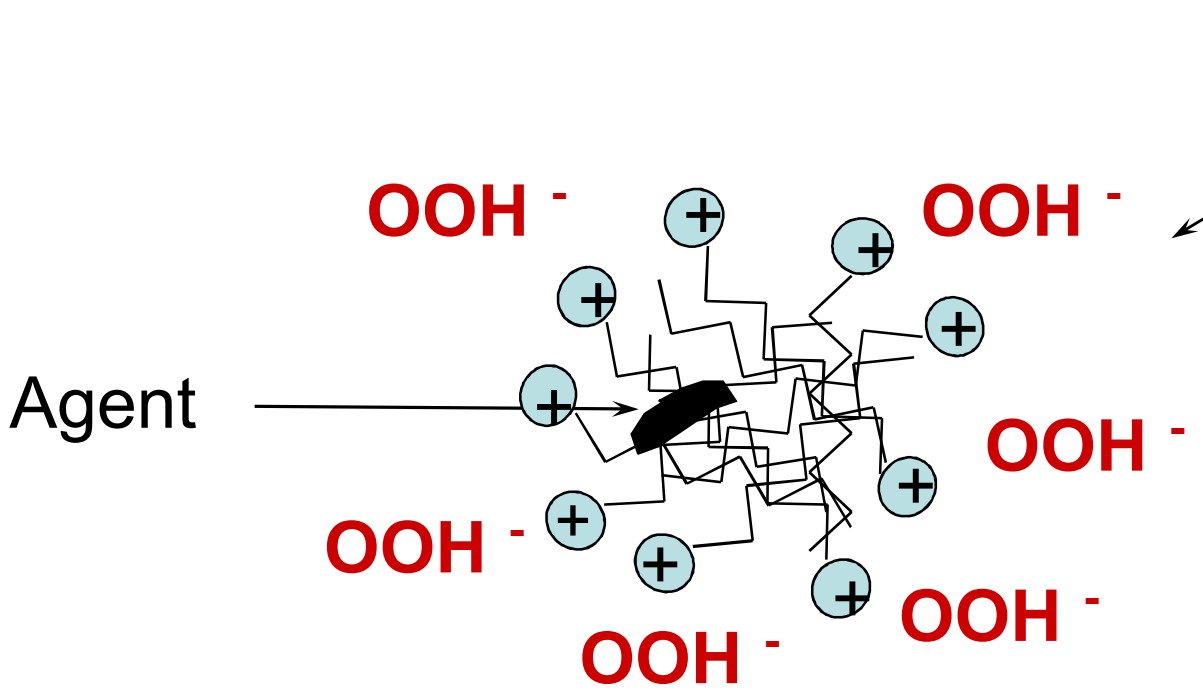
Micellar Catalysis with a Mixed Reactant System

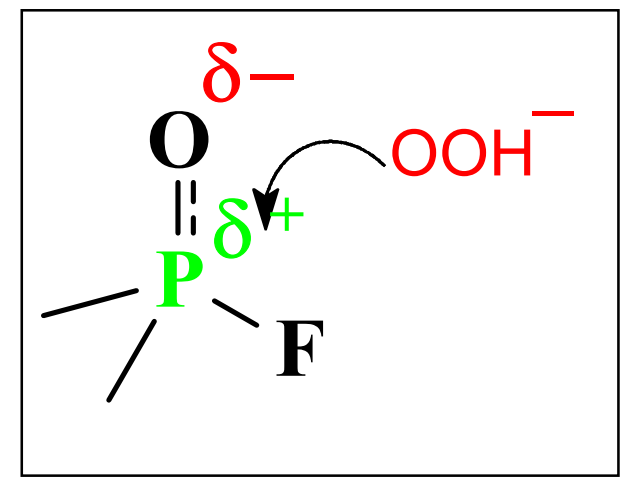
Activated Peroxide System

1. $H_2O_2 \rightarrow OOH^- + H^+$

2. $OOH^- + HCO_3^- \rightarrow HCO_4^- + OH^-$

3. 





Cationic micelle, catalysis

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Corrosion Comparisons

Steel Coupons

Deionized Water - 24 Hour Exposure

Sandia Decon Formulation - 24 Hour Exposure

Bleach - 24 Hour Exposure

The Sandia Decon Formulation (DF-200) was developed as an alternative to harsh formulations such as bleach

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Sandia Decon Foam (DF-200)

Summary of Decon Effectiveness

Agent	Contact Time (Minutes)	Byproducts
Nerve Agents (G)	1-10	Hydrolysis
Nerve Agents (V)	10-15	Breakage of P-S Bond
Vesicants (HD)	30	Oxidation
Anthrax Spores	15-30	Killed Organisms
Vegetative Bacteria, Viruses	1-10	Killed Organisms

Application Rate: 0.5 L of DF-200 Liquid per square meter of contaminated surface

Challenge Level (CW): 10 g of agent per square meter

Challenge Level (BW): 10^7 organisms per square centimeter

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Efficacy of Sandia DF-200 Formulation against CW Agents (US DoD Testing)

Decontaminant	GD		VX		HD	
	10 Min.	60 Min.	10 Min.	60 Min.	10 Min.	60 Min.
DS2	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
DF-200	>99.9	>99.9	97.8	>99.9	84.8	99.9

Percent decontamination from kinetic tests against CW agents (US DoD stirred reactor tests using EasyDECON™-200 Lot 3829 at 25˚C).

DF-200 has also successfully neutralized many toxic chemicals such as hydrogen cyanide, sodium cyanide, phosgene, carbon disulfide, malathion, tamoxifen, and capsaicin

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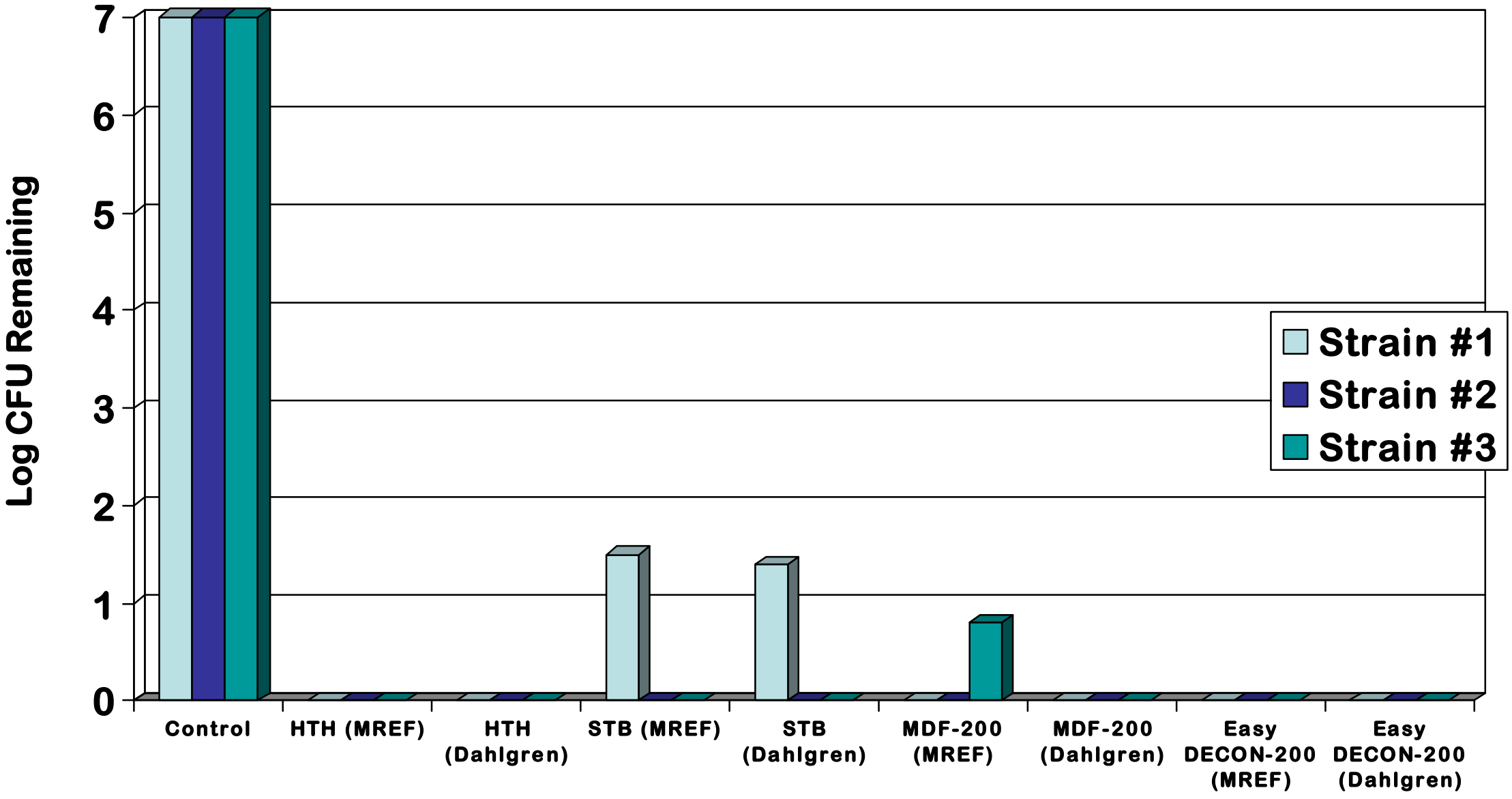
Efficacy of Sandia DF-200 Formulation against Three Strains of Anthrax Spores (US DoD Testing)

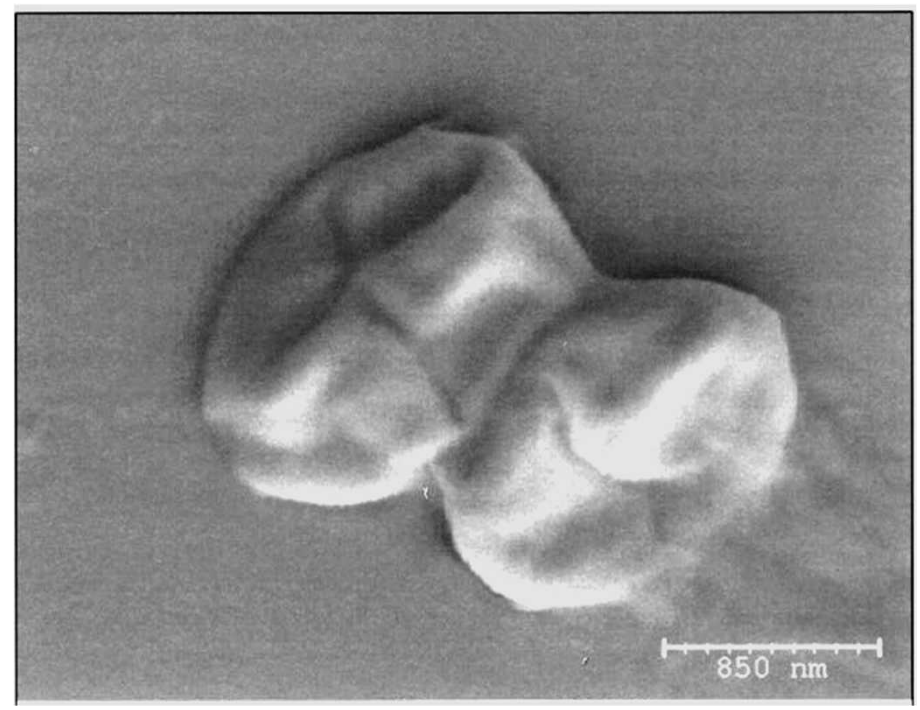


Residual spores following decontamination of CARC coupons (Initial surface loading: 1 x 10^7 spores; Contact time: 30 minutes; coupons rinsed in sterile de-ionized water following testing)

Kill of Anthrax Simulant

Scanning Electron Microscopy

As received



Sandia Foam
Contact Time: 1 Hour

Destruction of bacterial spores in Sandia foam (3.6% Hydrogen Peroxide)

Normally – it requires ~15% hydrogen peroxide to kill bacterial spores

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EPA Registration Received or in Progress under FIFRA

Commercial Versions of DF-200 (MDF-200™, EasyDECON-200™)

Fungus

- *Trichophyton mentagrophytes*
- *Aspergillus niger*
- *Penicillium variable*
- *Stachybotrys chartarum*

Viruses

- Influenza A
- Influenza B
- Norovirus (Feline Calicivirus surrogate)

Bacteria

- *Staphylococcus aureus*
- Methicillin resistant *Staphylococcus aureus* (MRSA)
- *Staphylococcus aureus* - VISA
- *Staphylococcus epidermidis*
- *Salmonella enterica* (choleraesuis)
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Proteus mirabilis*
- Vancomycin Resistant *Enterococcus faecalis*
- *Enterobacter aerogenes*
- *Escherichia coli* (0157:h7 and ESBL)
- *Listeria monocytogenes*
- *Clostridium difficile* (vegetative)

DF-200 based products are also highly effective against biofilms

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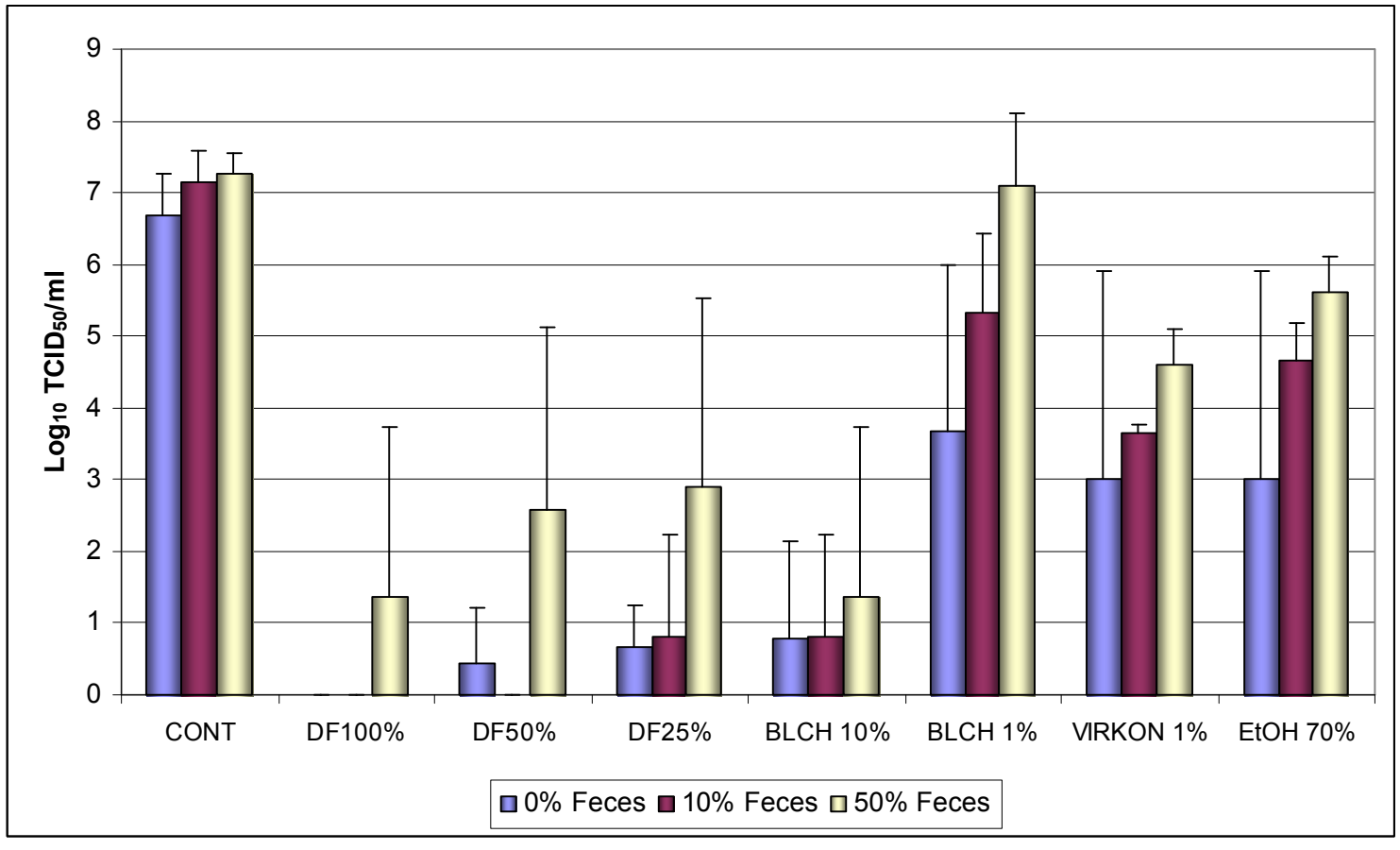
Inactivation of Avian Influenza Viruses

- Test disinfectants were evaluated at KSU for efficacy against isolates of influenza A including a mammalian strain (H1N1, A/WSN/33) and a low pathogenic strain (H5N8, isolated from turkey) which are surrogates for H5N1.
- Disinfectants tested include ethanol (70%), bleach (1, 10%), Virkon® S (1%), and DF-200, diluted by a factor of 2. This formulation was designated as DF-200D and was tested at 25, 50, 100% strength.
- Infectious titer was determined in $TCID_{50}$ format following treatment with the test disinfectants at various organic loadings.



Infectious titer of mammalian test isolate A/WSN/33 H1N1 determined by TCID50 following 1 minute exposure. CONT, Positive control; DF 100%, DF-200D (100%); DF 50%, DF-200D (50%); DF 25%, DF-200D (25%); BLCH 10%, bleach (10%); BLCH 1%, bleach (1%); VIRKON 1%, Virkon S (1%); EtOH 70%, ethanol (70%)

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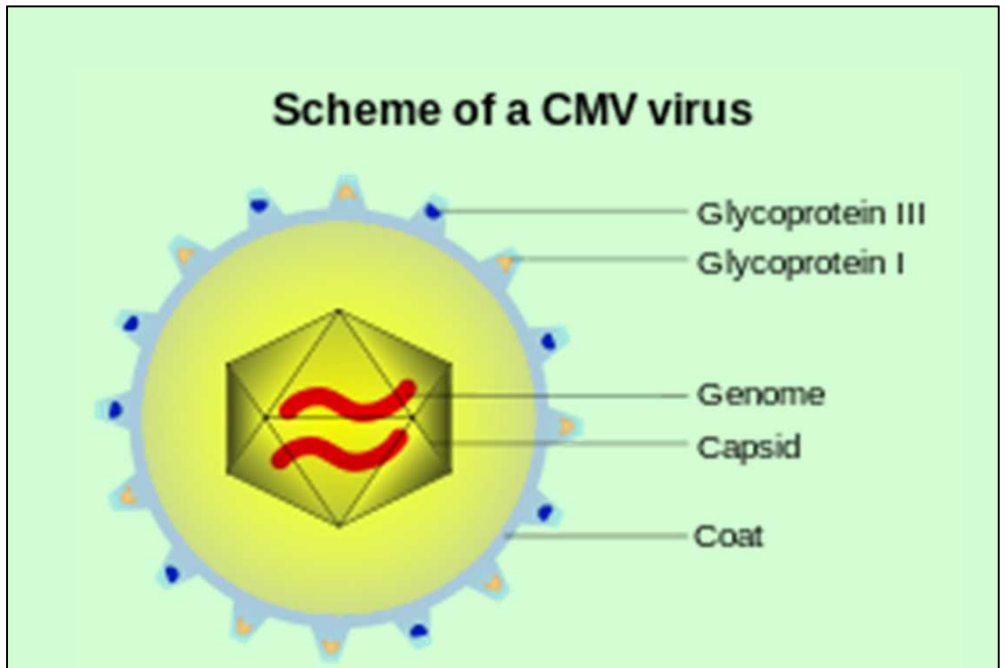
Challenges in the Disinfection of the Ebola Virus



The presence of the viral envelope makes Ebola easier to inactivate as compared to non-enveloped viruses

In very limited studies, the Ebola virus may remain active in the environment for several days (in the presence of bodily fluids)

Ebola patients may discharge up to 7 liters of bodily fluids per day (with active virus)

There are no products currently registered by US EPA against the Ebola virus

A strong disinfectant that remains active in the presence of organic loading (i.e., bodily fluids) may give the highest efficacy

Images from Wikipedia

Mold Remediation

Field Deployment of MDF-500™

Before

After

The Sandia Decon Foam has commercial, industrial, and military uses

Sanitization of Hospitals and other Public Areas

Homeland Security

Meth Lab Clean-up

Commercial, Industrial, and Military Uses of the Sandia Decon Foam

Mold Remediation

Pesticide Neutralizer

Military Use

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Decontamination of complex interior spaces is difficult

Complex Interior Spaces

Objective

Develop approach for aerosol delivery of decontaminants

<u>Liquids</u>
Oxidants, enzymes, solvents

Advantage: Agent-specific, can be non-corrosive

Disadvantage: difficult to reach all surfaces

<u>Gases</u>
ClO_2, Ozone, EtO

Advantage: Can reach all surfaces

Disadvantage: all known gases are toxic and/or corrosive

<u>Vapors</u>
VHP, mVHP

Advantage: Reaches all surfaces under certain conditions

Disadvantage: too corrosive for many interior spaces

<u>Aerosols</u>
Oxidants,enzymes, solvents

Advantage: Agent-specific, can be non-corrosive; reactivity

Disadvantage: need controlled conditions to reach all surfaces

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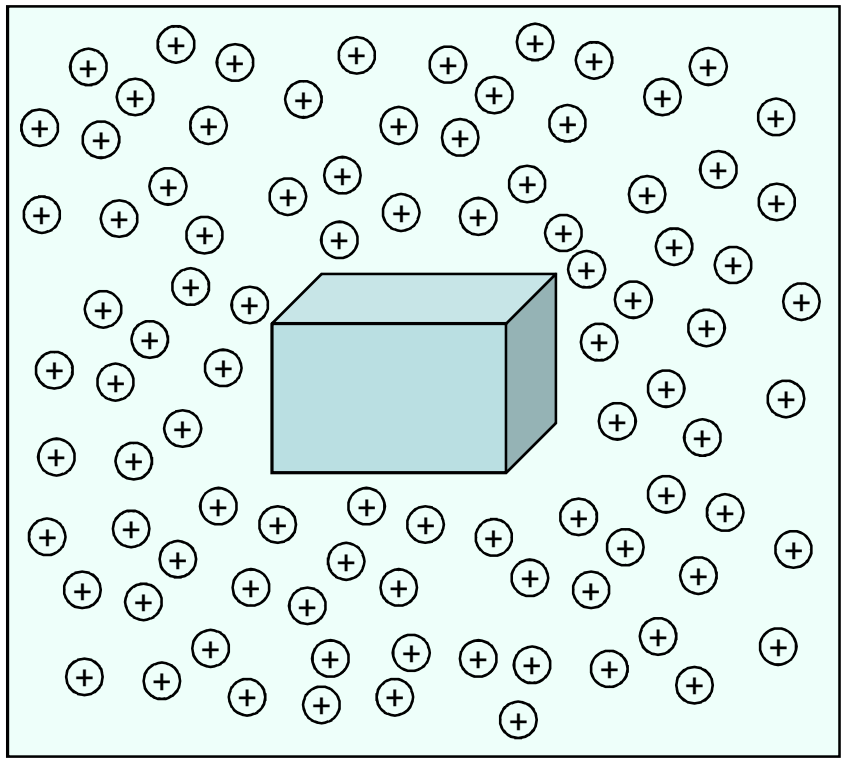
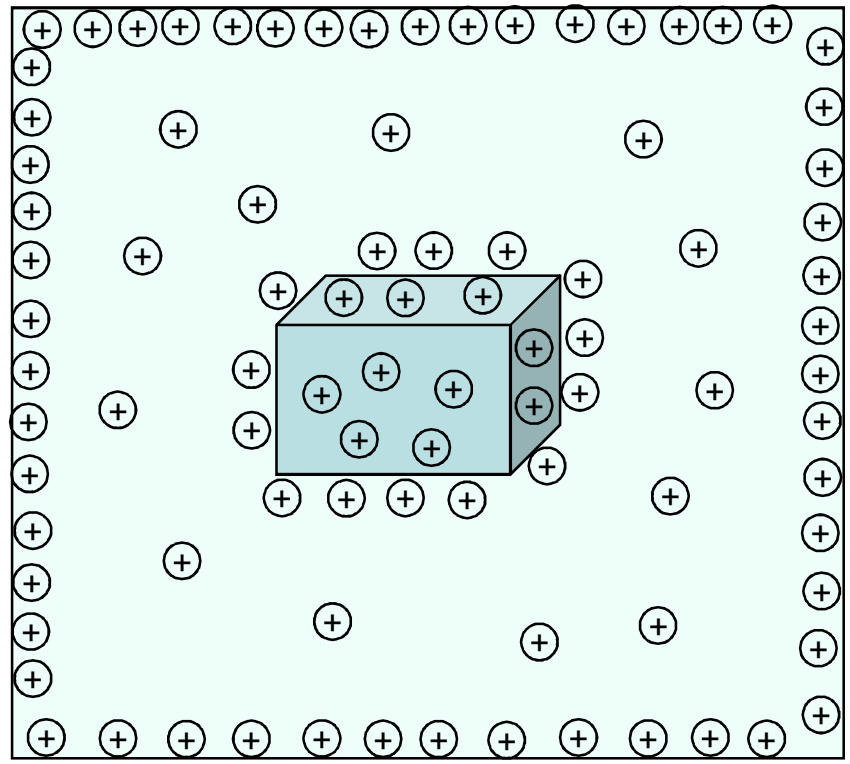
Use of the space charge effect can significantly enhance aerosol transport and surface coverage

Airborne charged droplets in a space



Charged droplets forced to surfaces by space charges



For uniform deposition on exposed and hidden surfaces we need:

- **Droplet size small enough to remain airborne during convection to hidden surfaces**
- **High enough droplet charge (charge to mass ratio) to make electrostatic forces dominant**
- **High enough droplet concentration to provide sufficient space charge to drive deposition**

A rotary atomizer was found to be the best device for dispersal of liquid decontaminants

Rotary atomizer for aerosol delivery of decontaminants

Test chamber

Advantages of this technology include: 1) Droplet size (~3-5 µm), 2) Ability to charge droplets, and 3) Uses a mechanical process to create droplets so it does not require large volume of air.

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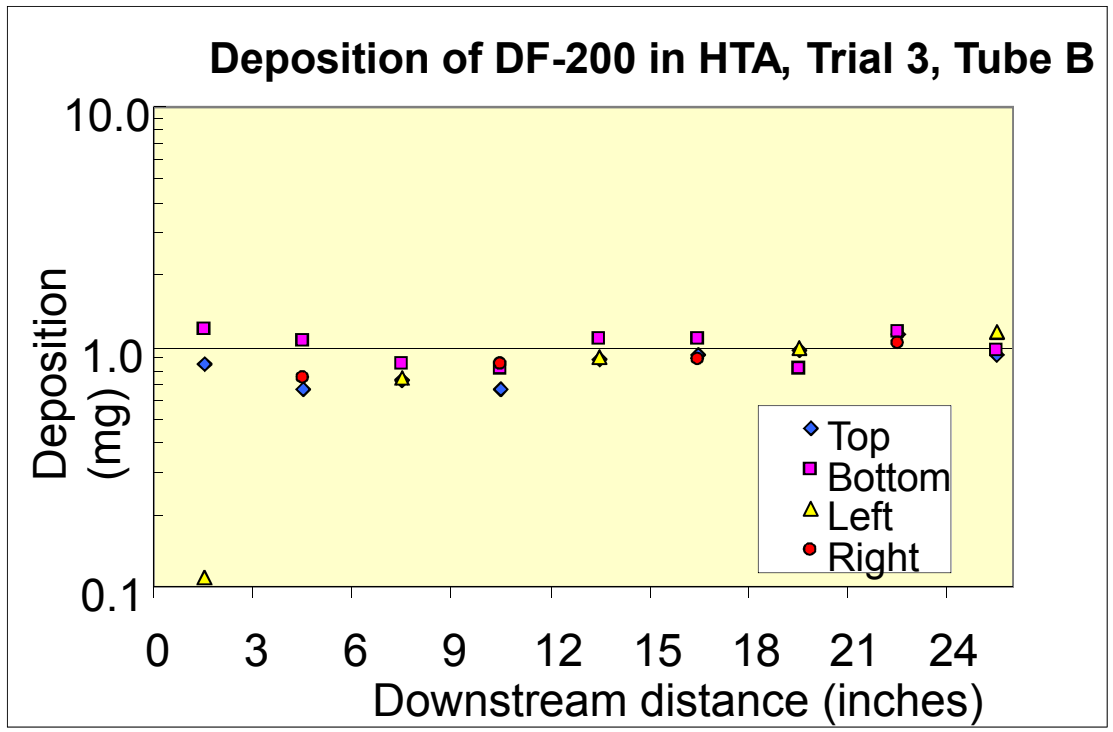
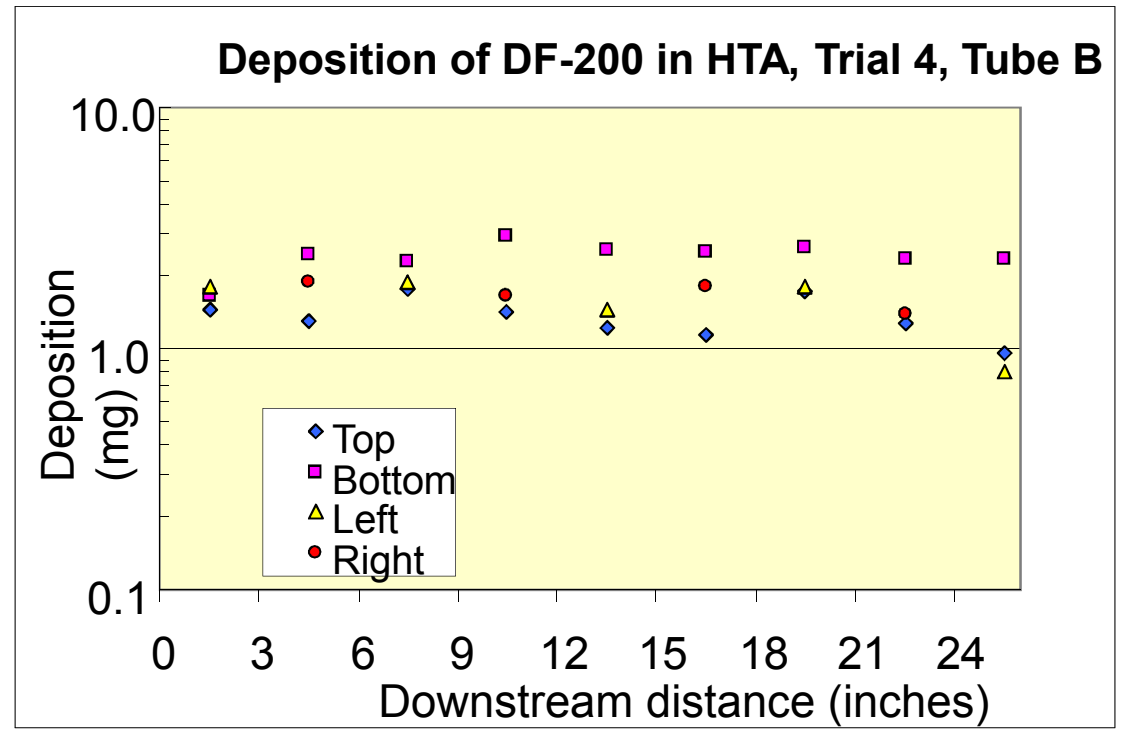
Example of experimental results using the rotary atomizer aerosol generation device





Aerosol generation conditions (i.e., charge and concentration) were varied until nearly uniform deposition was achieved on all surface orientations and down the length of the hamster tubes

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Following the selection of an aerosol generation device, we investigated decontamination methods using the device

- Application of a modified DF-200 formulation (for both CW and BW agent surrogates)
- Application of a two-step decontamination process for bacterial spores
  - Aerosol dispersal of a germination solution
  - Aerosol dispersal of a mild “kill” solution
- Application of other liquid decontaminants for both CWA and BWA decontamination (collaboration with Boeing – in progress)
  - Peracetic acid
  - Enzymatic

Protocol for Bio-Efficacy Tests

Test Parameters

- *Bacillus globigii* spores on stainless steel biological indicators were used (spore loading: 10^6)
- A constant DF-200 flow rate to the proprietary aerosol-generation device was used
- The trials ran for 120 minutes or 240 minutes
- The exhaust flow was set at 75 CFM
- Only the droplet charge was changed between runs
- Timed samples were collected
- Samples were neutralized with thiosulfate when collected

Liquid coverage of DF-200:

- ***Target for Foam Applications (Surface Decon)***: 5 mg/cm^2 for 30 minutes
- ***Target for Aerosol Applications***: 0.05 to 0.5 mg/cm^2 for 60 minutes
- ***Comparison***: a typical paint application is 10 mg/cm^2 (400 ft^2 per gallon)

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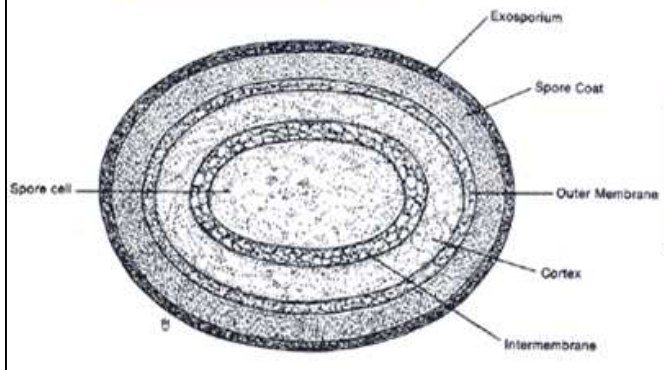
We have investigated a non-toxic, low-corrosivity decon method to kill highly resistant bacterial spores in complex interior spaces

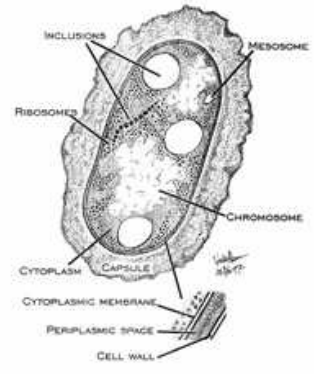
- A chemical solution that triggers the germination process in bacterial spores and causes those spores to rapidly and completely change to much less-resistant vegetative cells that can be easily killed.
- Vegetative cells are then exposed to mild chemicals (e.g., low concentrations of hydrogen peroxide,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

Bacterial Spore



Activation and Germination

Vegetative Cell



To kill spores…

- 15% Hydrogen Peroxide
- 10% Bleach
- Chlorine Dioxide
- Paraformaldehyde
- Ethylene Oxide

To kill vegetative cells…

- 1% Hydrogen Peroxide
- 0.5% Bleach
- Alcohol
- Quats
- Non-toxic Aldehydes

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

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Initial Test Results

- **Our process employs a novel germination solution consisting of low-cost, non-toxic and non-corrosive chemicals.**
- **We have tested both direct surface application and charged-aerosol delivery of the solutions.**

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

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

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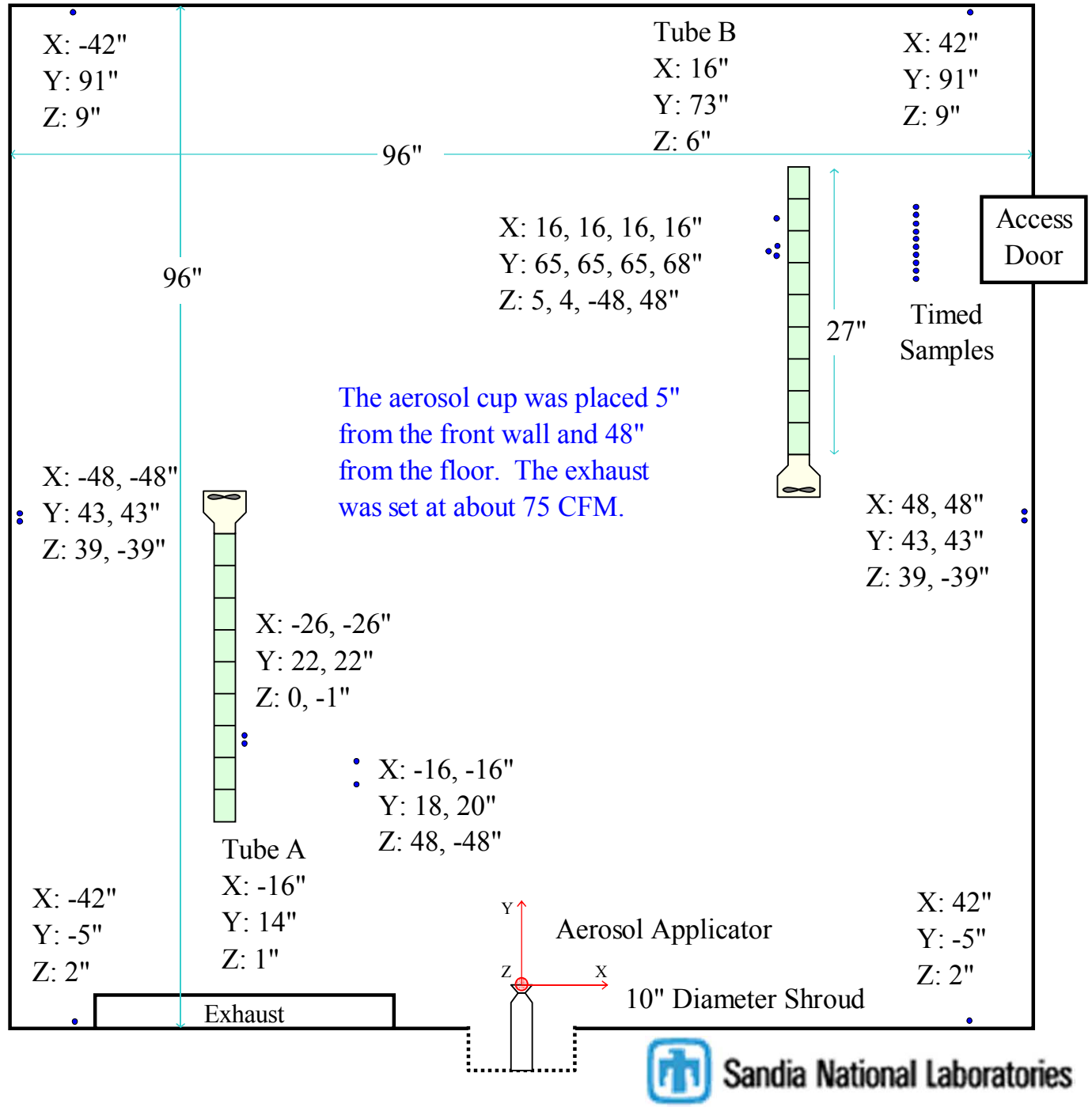
Coupon Placement for Bio-Efficacy Tests

Coupon Placement

- 8 coupons on the walls.
- 2 coupons on the ceiling and 2 coupons on the floor.
- 2 coupons on top of the platforms supporting the hamster tubes.
- 2 additional coupons at various positions in the chamber.
- 8 timed samples not in the line-of-sight of the aerosol (bio-tests).
- 10 coupons in each hamster tube (bio-tests).
- 10 coupon controls (bio-tests)

Test Chamber (Plan View)



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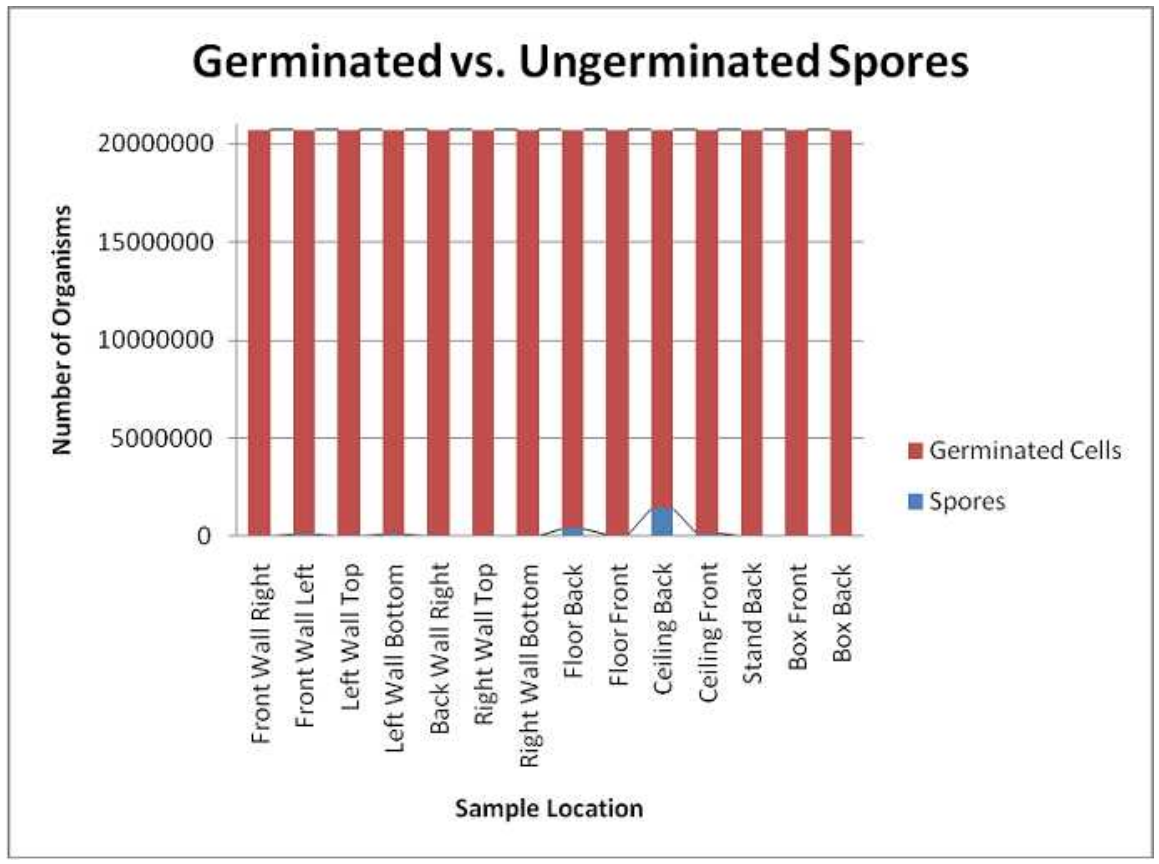
Summary of rapid germination results

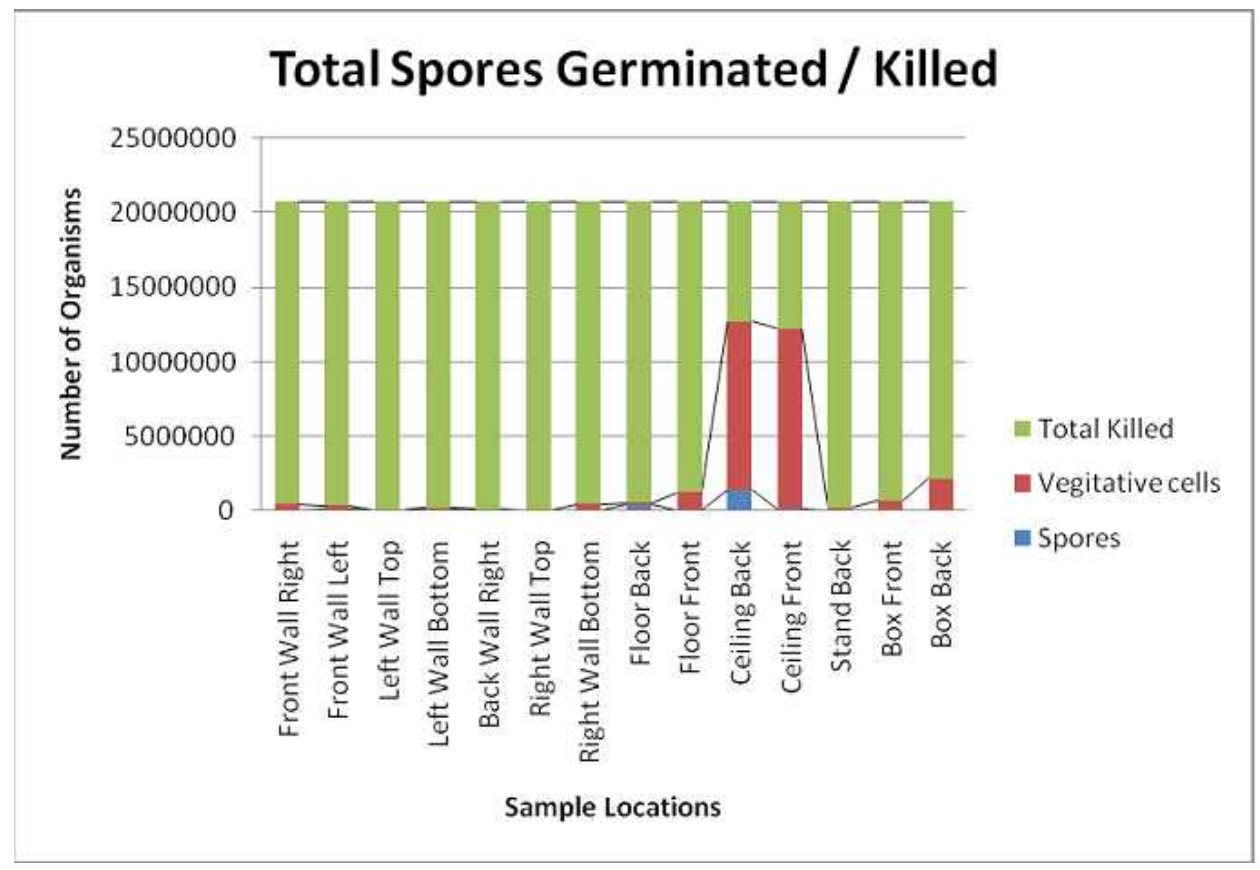
Red = germinated spores
Blue = ungerminated spores



“Kill” solution was 3% hydrogen peroxide

Germination solution deployment → **Wait** → **“Kill” solution deployment**

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



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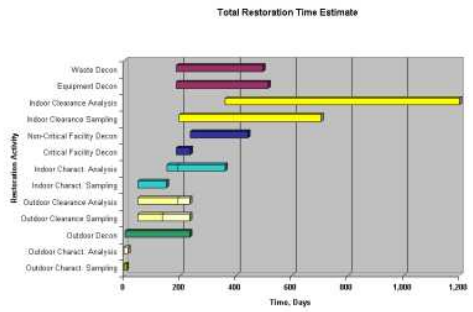
Sandia has broad expertise in applying a systems approach to post-event consequence management

Formal Response & Recovery Guidance and Decision Tree Development



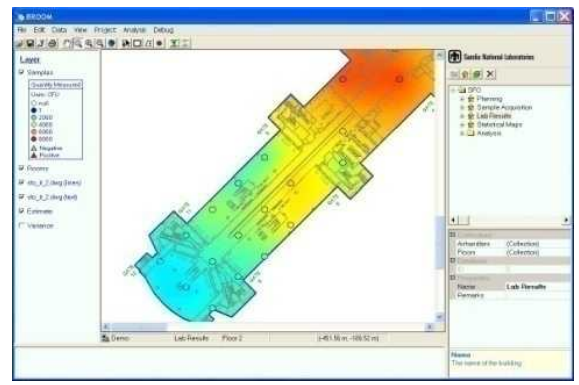
Remediation Planning and Decision Tools

Blast experiments

Strippable gel for rad decon

Aerosol experimental chambers

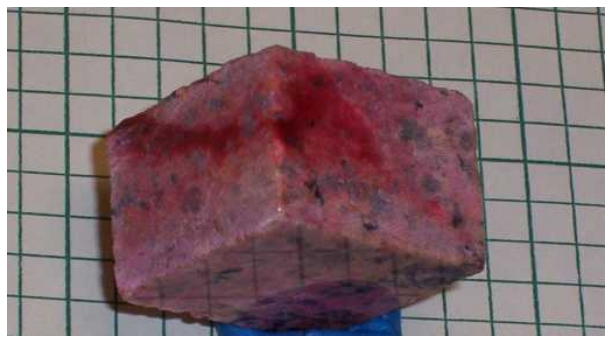
Decontamination technologies



Real-time contamination mapping and sampling decision tools

Systems studies of CB and dirty bomb threats

NISAC tools for infrastructure analysis



Surface sorption experiments

Sandia's broad experience assures a comprehensive, systems-level solution