U.S. Department of Energy

Chemical & Biological Nonproliferation Program FY99 Annual Report

CHEMICAL & BIOLOGICAL NONPROLIFERATION PROGRAM • DOE

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The threat of an attack with chemical or biological weapons on U.S. citizens is a high priority concern. In the face of such threats, our national security is increasingly defined by our ability to develop and apply new tools that take advantage of recent advances in technology. As part of a government-wide effort, the Department of Energy (DOE) Chemical and Biological Nonproliferation Program (CBNP) is developing new capabilities to counter the chemical and biological threat.

Within the CBNP, we are engaging the best and the brightest at the national laboratories and elsewhere to develop advanced technological solutions to the chemical and biological threat. At its core, the DOE is a science and technology agency. The Department's work in the biological sciences began with early studies of the effects of radiation on the human body and continues today with programs to develop new biological diagnostics and to sequence the human genome. Our biological expertise combined with important capabilities in chemistry, modeling and simulation, and relevant engineering sciences form the basis of our efforts to counter the chemical and biological threat.

This document is the first of what will become an annual report documenting the progress made by the CBNP. It is intended to be a summary of the program's activities that will be of interest to both policy and technical audiences. This report and the annual CBNP Summer Review Meeting are important vehicles for communication with the broader chemical and biological defense and nonproliferation communities. The Chemical and Biological Nonproliferation Program Strategic Plan is also available and provides additional detail on the program's context and goals.

The body of the report consists of an overview of the program's philosophy, goals and recent progress in the major program areas. In addition, an appendix is provided with more detailed project summaries that will be of interest to the technical community.

We invite your comments on this report or the program in general.

Rose E. Gottemoeller
Acting Deputy Administrator for
Defense Nuclear Nonproliferation
March 2000

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The Department of Energy (DOE) Chemical and Biological Nonproliferation Program (CBNP) is an applied research and development program that focuses emerging science and technology on the challenging threat of chemical and biological weapon attack against civilian populations.

The CBNP was initiated in FY97 in response to the growing awareness of both the chemical and biological weapon capabilities available to potential attackers and the vulnerabilities of the U.S. population to these weapons. From the outset, the program has drawn primarily from the deep scientific and engineering talent in the DOE national laboratories, but it is also using specialized expertise in academia and industry.

Mission Focus

A basic philosophy of the CBNP is that any near-term impact of R&D requires a mission focus. The CBNP mission is to develop, demonstrate and deliver technologies and systems to improve domestic defense capabilities, and ultimately save lives in the event of a chemical or biological attack. In the civilian context, formal requirements have not yet been developed. In lieu of such requirements, the program emphasizes the use of analytical studies and system prototype development and demonstration to identify areas where technology can have the highest impact.

One major study, the Defense of Cities Study, was initiated in FY99 in collaboration with the DoD Defense Threat Reduction Agency to examine alternative system concepts for defending cities against chemical or biological attack. This study has established a framework for assessing the value of technology within an overall defense system. In particular, the important role of detection, identification and warning systems to cue both medical and protection responses was identified. This ongoing study will be important in guiding efficient resource allocations, both within the CBNP and potentially more broadly.

Another vehicle used by the CBNP to provide mission focus is the Domestic Demonstration and Application Program (DDAP), a concept analogous to the DoD Advanced Concept Technology Demonstration (ACTD) model. The goal of the DDAPs is to develop and demonstrate prototype systems for specific applications. This allows system operators to evaluate the technology and the overall system, and in turn clarifies the required performance levels of the system. Two DDAPs are currently underway:

- PROTECT: Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism.

- BASIS: Biological Aerosol Sentry and Information System.

Both are aimed at demonstrating integrated detection and warning systems; for high-threat infrastructure (initially subways and airports) in the case of PROTECT, and for special events such as political gatherings or sporting events in the case of BASIS. DDAPs are a critical element in the CBNP strategy to transition technology from the laboratory to operational use.

Major progress was made in FY99, in both PROTECT and BASIS, in developing the requirements of the systems as well as more detailed system concepts. In addition, the PROTECT team initiated evaluation of chemical detectors for use in a subway detector test-bed at the Washington, DC Metro system and conducted smoke releases to better understand the complex air flows in the subway environment. As part of the BASIS program, decision support tools were deployed during the Westwind exercise in support of the Los Angeles County Operational Area Terrorism Early Warning Group.

Enabling Technology

The DDAPs focus on transitioning technology into operational use in the near term (2–3 years). However, the threat of chemical and biological attacks is a long-term challenge. The CBNP R&D efforts are addressing this challenge. Current areas of active R&D include: detection, development of the biological foundations needed to support detection and treatment development, modeling of aerosol dissemination, and decontamination. The goal of these efforts is to yield prototype capabilities in the 3–5 year time frame.

The accomplishments in these areas over the past year have been impressive and are documented in detail in this report. A few examples:

Firefighters, law enforcement, facility operators, and medical emergency personnel need low-cost, sensitive, low maintenance, autonomous or easily portable detection tools that can detect a broad range of agents rapidly and without false alarms. Significant progress has been made in several CBNP detection projects towards developing detectors with these attributes. For example, the Autonomous Pathogen Detection System successfully operated autonomously for more than 12 hours in a wind tunnel, while being challenged with a pathogen surrogate.

In the biological foundations area, CBNP progress in improving U.S. capabilities to detect and analyze an incident of biological terrorism has resulted in analysis techniques being transferred to the agencies with primary operational responsibility. For example, the strain typing and phylogenetic characterization of B. anthracis and its relatives achieved by the CBNP is, to date, the most complete available and has been transferred to the Centers for Disease Control and Prevention (CDC).

The modeling and prediction capability being developed is unique and will provide users in intelligence, law enforcement and emergency management a vastly improved and integrated set of modeling tools for planning and response. Over the past year, physical processes important to describing the flow of chemical and biological aerosols

(e.g., particle deposition, surface energy transfer and vegetative canopy effects) were added to the models. In addition, initial progress was made in the important yet very complex area of model validation. For example, subway model predictions were compared to data obtained from experiments done in the New York City subway system in the 1960s.

New foam- and gel-based technologies being developed will allow the rapid and safe decontamination of facilities in the event of a chemical or biological attack. In the past year, reactive foams and gels have been tested on live chemical and biological agents and shown to be rapid and effective decontaminants. In addition, a novel approach based on atmospheric pressure plasma technology was tested on chemical agents and biological surrogates. This dry, nondestructive technique has potential for decontaminating sensitive equipment.

The progress achieved during FY99 described in this report illustrates that the investments made since FY97 are beginning to yield tangible results. In the coming years, the CBNP will continue to demonstrate advances in individual technologies and will increasingly facilitate the integration of these, as well as technologies being developed by other government programs and industry, into operational applications.

The scope of creating comprehensive capabilities to counter the use of chemical and biological weapons is enormous—such capabilities will not be developed by the CBNP alone. The program will continue to coordinate closely with other responsible federal agencies, including the Departments of Justice, Health and Human Services and Defense. In addition, through demonstrations and interactions with local authorities, the program is committed to understanding and developing the technology needs of those who will be on the front line if a chemical or biological attack occurs.

Background

The Department of Energy Chemical and Biological Nonproliferation Program (CBNP) was initiated in FY97 to engage the DOE and its laboratories more fully in the development and demonstration of new capabilities to improve U.S. domestic preparedness and response capabilities to chemical and biological attacks.

DOE and the national laboratories have a long history of supporting nonproliferation and national security policy. As part of its primary nuclear science and technology mission, DOE has developed extensive capabilities in chemistry, biology, materials and engineering sciences, and system engineering at its laboratories. These capabilities—in areas such as genomic sequencing, development of new DNA-based diagnostics, advanced modeling and simulation, and microfabrication technologies, as well as the nexus of these capabilities with expertise in nonproliferation and national security—form the basis for DOE's role in combating the chemical and biological threat. In addition to the chemical

First responders attending to victims after the 1995 sarin attack on the Tokyo subway.

and biological nonproliferation activities supported by this program, the national laboratories conduct over $50 million per year in chemical and biological defense research for other government agencies.

The Chemical and Biological Threat Poses a Complex Defense Challenge

Technology plays a critical role in defending the U.S. population against attack(s) with chemical and biological weapons. These emerging threats, whether of domestic or foreign origin, are rooted in science and technology and any effective response must draw on similar expertise. However, technology is only one dimension of the complex system

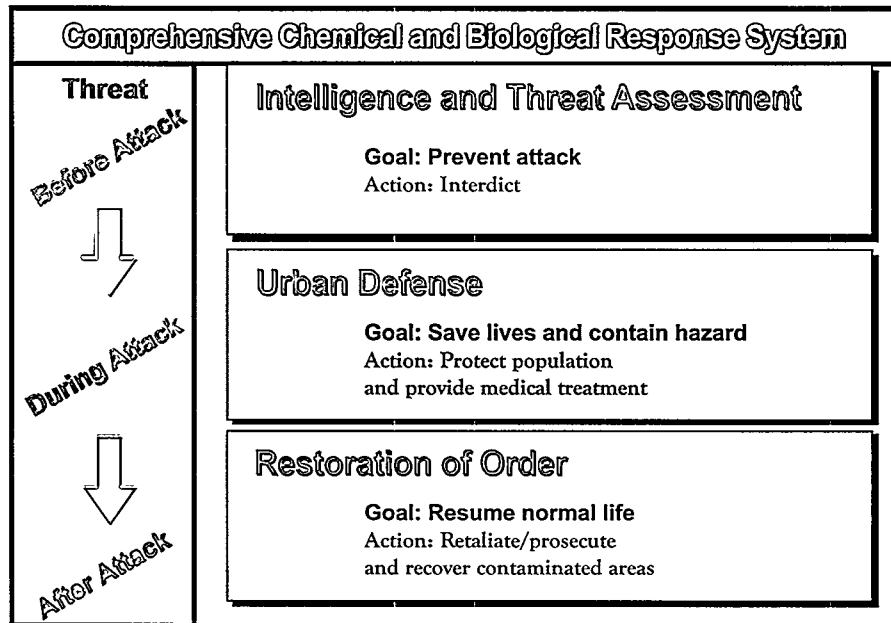**A terrorist at the 1972 Olympic Games in Munich.**

An explosion near a crowded area of the 1996 Olympic Games in Atlanta.

of people, organizations and policies, operational procedures, physical resources, and information flow that comprises a preparedness and response capability. Technology must be developed within this context to effectively anticipate and meet operational needs.

Comprehensive Chemical and Biological Response System

Threat

Before Attack

During Attack

After Attack

Intelligence and Threat Assessment

Goal: Prevent attack
Action: Interdict

Urban Defense

Goal: Save lives and contain hazard
Action: Protect population
and provide medical treatment

Restoration of Order

Goal: Resume normal life
Action: Retaliate/prosecute
and recover contaminated areas

The emphasis, goals and actions of an overall response system depend on the phase of attack—before, during, or after—being addressed.

The Comprehensive Chemical and Biological Response System figure above shows the elements necessary to prepare for and respond to the chemical and biological threat. These elements include:

Intelligence and Threat Assessment
- Intelligence on suspect activity.
- Interdiction to prevent an attack.

Urban Defense
- Detection that an attack has taken place.
- Protection and medical systems to reduce casualties.
- Command and control systems for managing the defense.

Restoration of Order
- Attribution of responsibility and prosecution or retaliation.
- Restoration of contaminated facilities or areas.

The primary focus of the CBNP is the *Urban Defense* element of the Comprehensive Response System. This element is discussed in more depth following a brief sketch of the other two elements, *Intelligence and Threat Assessment* and *Restoration of Order*.

Intelligence and Threat Assessment
In general, the earlier that the threat can be managed, the more effective are efforts toward defense. Timely and accurate intelligence, if available, is extremely valuable and can lead to interdiction by law enforcement or military forces to prevent a planned attack from taking place. However, obtaining timely and accurate intelligence is a formidable challenge due to the dual-use nature of much of the relevant technology and the relatively small quantity of materials required to inflict mass casualties.

Restoration of Order

If an attack is successfully executed, attribution of responsibility is necessary for subsequent retaliation or prosecution. Attribution can be very difficult in small-scale attacks or even in large-scale attacks involving covertly delivered biological agents that can take days to produce symptoms.

Urban Defense

The Urban Defense element of the Comprehensive Response System is detailed in the Chemical and Biological Urban Defense System figure below. The goal of this element is to minimize casualties in the event of an attack. This element is the primary focus of the DOE CBNP, although some gaps in the other two elements areas are also being addressed.

A focus on Urban Defense resulted from a process that identified needs in the functional areas of the comprehensive response system described above and matched those needs to the strengths of the DOE national laboratories. The CBNP Strategic Plan, available as a separate document, summarizes the needs identified through the process.

Scanning electron micrographs of *Bacillus anthracis* and *Yersinia pestis*.

The fundamental goal of the Urban Defense system is to prevent casualties in the event of a chemical or biological attack.
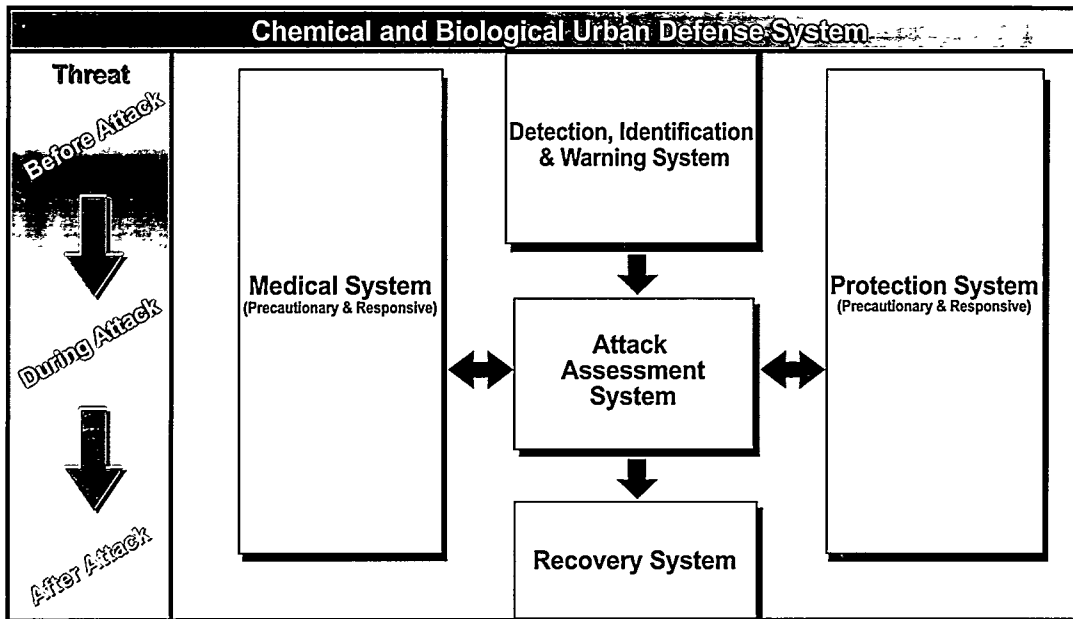
The *Urban Defense* element of the Comprehensive Response System is the transition between *Intelligence and Threat Assessment* and *Restoration of Order*. It consists of five subsystems, together with the information flow among them (denoted by arrows).
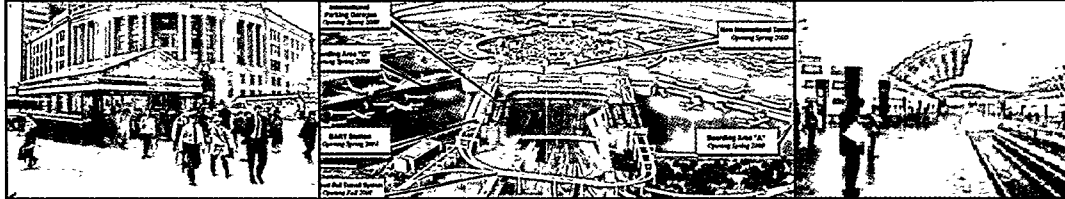
Defending cities from a chemical or biological attack necessarily involves evaluating vulnerability of and response options for subway systems and airports.

To achieve this goal, it is critical to have timely detection, identification, and warning that can cue both medical and protection responses; hence, a major emphasis of the CBNP is the development of detection technology to provide such cues. More generally, there are important roles for technology, and therefore opportunities for research and development contributions, in each of the five major system components shown in the Urban Defense system figure:

- Detection, Identification and Warning (DI&W) System.
- Attack Assessment System.
- Protection System.
- Medical System.
- Recovery System.

The function of each component, along with their integration, is described in more detail in the Defense of Cities study overview in the next section.

This functional description of the Urban Defense system, while useful for evaluating the relative utility of various technologies, is not easily amenable to the management of a research and development program, which is more effectively organized around technical disciplines. The CBNP is directed at addressing the gaps in the functional elements, but is organized by technical area as

described below. Throughout this report, the impact of the technologies being developed on these functional areas will be indicated.

CBNP Organization

Guided by the goal of advancing technology that can be applied to the functional areas above, the CBNP has three principal elements:

- Analytical Studies.
- Technology Development.
- Domestic Demonstration and Application Programs (DDAPs).

Analytical Studies

The program relies on studies and analyses to help guide the overall program direction as well as individual technical areas. In general, these studies use analytical and simulation models to assess the value of technology in system applications. At the program level, such studies are useful in comparing impacts of the various technology development areas within the CBNP. They can also be directed at the more comprehensive response system that extends well beyond the scope of the CBNP Domestic Demonstration and Application Programs. One overarching study was initiated in FY99 to look at developing alternative system concepts for defending cities against chemical or biological attack.
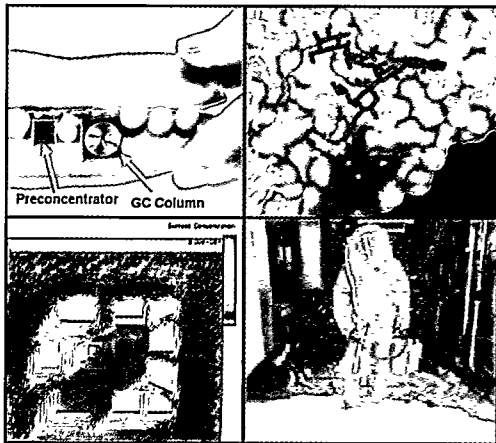
Clockwise from top left: microfabricated components for chemical and biological detection, visualization of a compound designed to block the function of a toxin, developmental foam for decontaminating equipment after exposure to a chemical or biological hazard, and output from a predictive model showing hazard levels around an office complex.

Technology Development Thrust Areas

Technology Development is the core program element. In general, development is focused on technologies for which the basic science is already understood. The goal is to yield prototype capabilities in the 3–5 year time frame. Currently there are four areas of specific focus:

- Detection.
- Biological Foundations.
- Modeling and Prediction.
- Decontamination and Restoration.

The DOE program is addressing R&D needs in only selected areas within the overall defense system architecture. For example, the program does not support R&D in medical treatment or individual protection (suits, masks), since other agencies have comprehensive programs in these areas. Coordination in those areas in which sever-

al agencies are pursuing R&D is essential and mechanisms for ensuring coordination are discussed in the External Interfaces and Coordination section.

Domestic Demonstration and Application Programs

The Domestic Demonstration and Application Programs (DDAPs) bring together individual technologies into more capable systems. This integration is important since it is usually only at the system level that problems are solved. The goal of the DDAPs is to integrate current technology into prototype operational systems directed at specific applications. The DDAPs also provide a vehicle for introducing emerging technology and limited capability systems into operational settings, giving system operators experience with the technology. In addition, DDAPs are a key mechanism for understanding the performance requirements that technology must satisfy. There are two DDAPs currently underway:

- PROTECT: Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism.

- BASIS: Biological Aerosol Sentry and Information System.

Both of these programs focus on the demonstration of early detection, identification, and warning (DI&W) systems. In addition, the two DDAPs will require the development of the interface between the detection system and the command and control systems that will ultimately take action based on the DI&W information.

Analytical Studies

Analytical studies play a key role in any effective R&D program. Often such studies are focused on a specific application of a technology or conducted as part of a DDAP. In FY99, the DOE CBNP, together with the Advanced System Concepts Office of the DoD's Defense Threat Reduction Agency, initiated a major study to take a broad look at the potential roles for technology in reducing casualties in the event of a chemical or biological attack on a city.
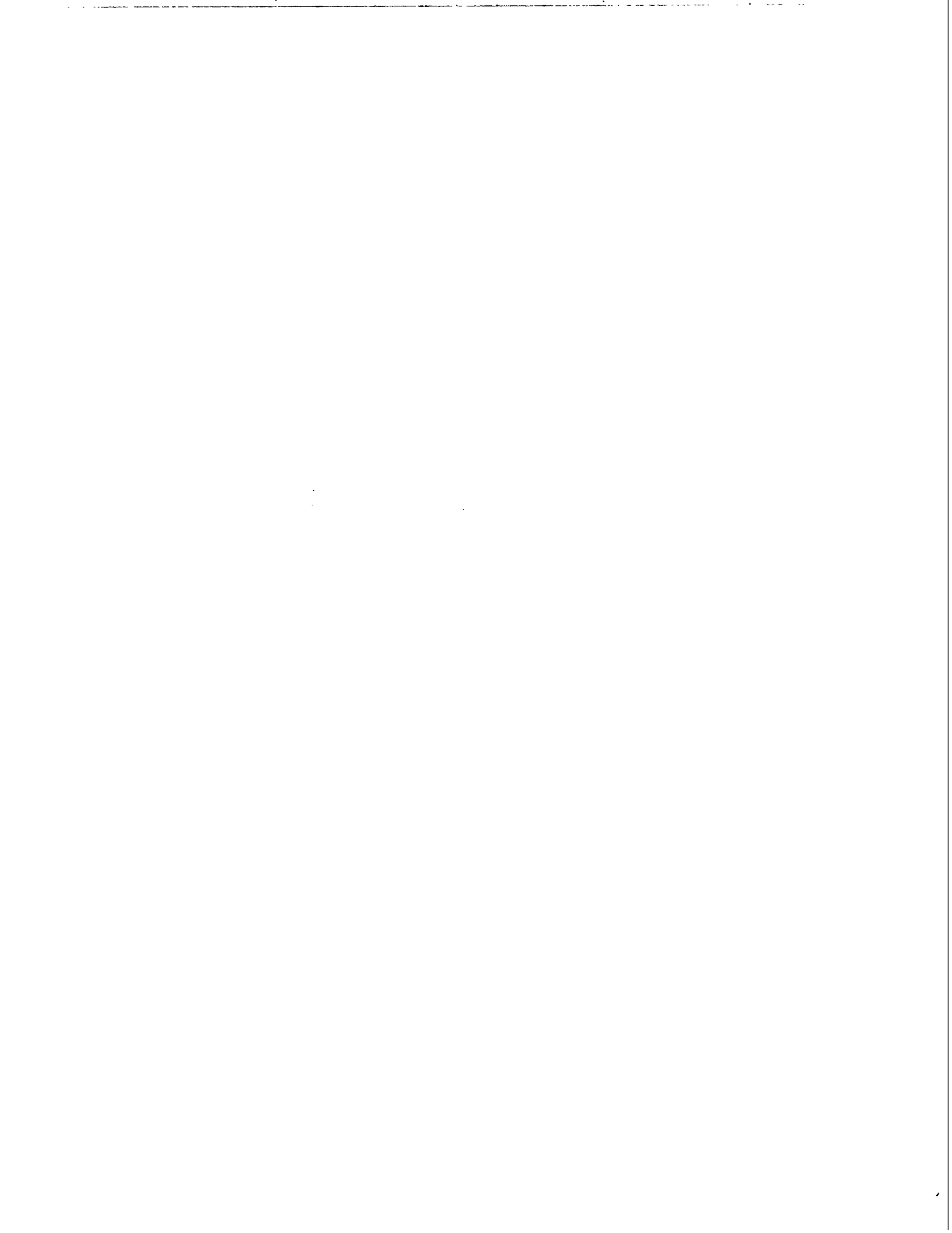
Objectives

This study examines how technology can be more effectively employed to make a significant contribution to reduction of population casualties resulting from attacks against U.S. cities with chemical and biological (CB) weapons. There are two primary objectives:

- Provide guidance to the CBNP to focus its resource investments by identifying cost-effective and high-leverage technologies.

- Contribute to the development of an effective national defense architecture in which the roles, responsibilities, and resources of the various involved agencies and jurisdictions are closely coordinated.

System Description

A top-level assessment of concepts and strategies for defense of cities against CB attack was undertaken. From this assessment, a candidate CB urban defense system was proposed that includes technology, operational concepts, and organizational structures. The candidate system is designed to operate in a city engaged in reasonably normal day-to-day activities prior to detection of a CB attack in progress. The major elements of the system are shown in the figure below.

The system is designed to operate without advanced warning of attack, but can also make use of such warning if available. The system architecture builds upon existing urban infrastructure and capabilities and is based on a "system-of-systems" approach that is composed of precautionary and responsive system elements. The primary system elements, illustrated in the figure below, are:

- Detection, Identification and Warning (DI&W) System.
- Attack Assessment System.
- Protection System.
- Medical System.
- Recovery System.

Detection, Identification & Warning (DI&W) System

This system provides critical data confirming that a chemical or biological agent is present in the environment. The primary sources of these data are (in increasing order of timeliness) medical surveillance, laboratory diagnostics of patient samples, and environmental sample collection and analysis.

Chemical and Biological Urban Defense System
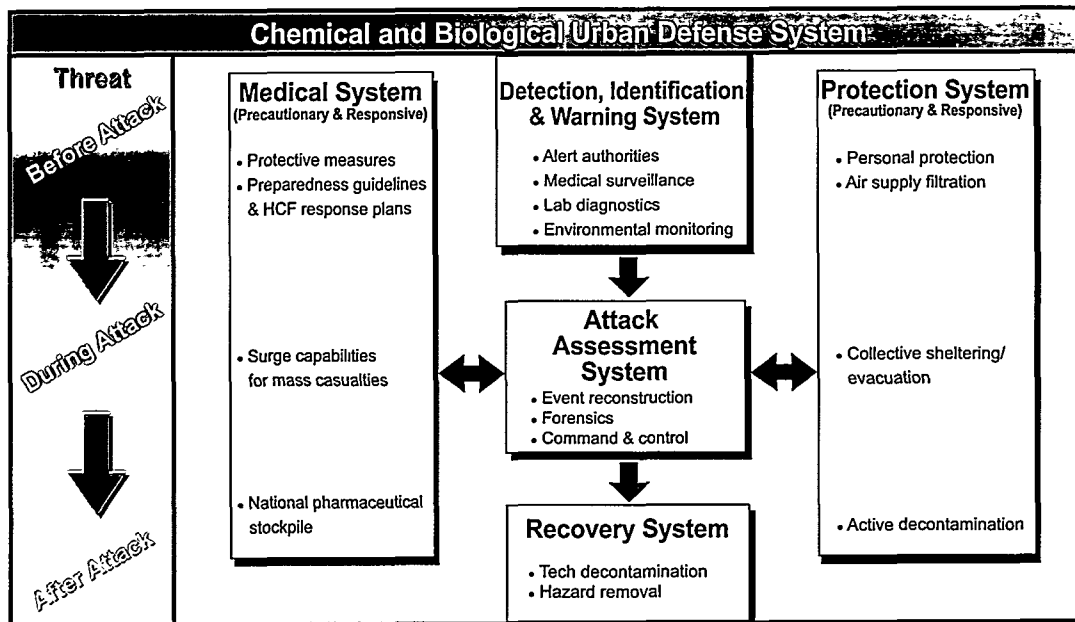
The primary value of the DI&W system is to reduce the time between an attack and the discovery that it has taken place in order to enable timely implementation of protection and medical responses.

Attack Assessment System

This function serves as the "central command" for the entire system. Based on information provided primarily by the DI&W system, attack assessment uses databases, information management systems and human analysis to produce the best possible understanding of the attack and potential consequences. Event reconstruction occurs here, resulting in an estimate of the nature and scope of the attack. The goals of event reconstruction are to direct effective responsive measures, both protection and medical, for casualty mitigation and to provide forensic clues to law enforcement.

Protection System

By definition, preventing exposure has a key role in reducing attack casualties, thereby reducing the burden on medical resources. Protective measures can be both precautionary (e.g., advanced filters in buildings) and responsive (e.g., collective sheltering/evacuation, air flow changes in facilities, train control in subways, masks and suits for individuals, and active decontamination). Responsive measures are triggered by the attack assessment function in order to be initiated.

Medical System

Even with highly capable DI&W, attack assessment, and protection systems, medical care will be required in the event of an actual attack. Like the protection system, the medical system has both precautionary (e.g., pharmaceutical stockpile deployment) and responsive elements (e.g., therapeutics administered post-exposure or post-symptoms, patient decontamination). Significant value is gained if medical resources can be cued in a timely manner; for example, in the case of anthrax, beginning treatment post-exposure, but pre-symptoms.

Recovery System

In addition to managing the human casualties resulting from an attack, normal activity can only be resumed if all hazard areas, whether indoor or outdoor, are rendered free of contamination. Technical measures, such as applying decontaminating materials to affected surfaces, are required.

FY99 Highlights

Because the outcomes of a CB attack are subject to a wide range of uncertainties, analysis of the contributions of defense system elements to protecting urban populations is problematic. In order to deal with these uncertainties and assess the potential contributions of technologies and systems, the project team developed a model that integrates non-medical and medical considerations into a complete portrayal of events and results from agent release to outcome. This model is based on the concept of *master timeline curves*, which provide a means of illustrating the effects and outcomes associated with CB attack under a range of scenarios employing various agents. The curves depict, as a function of time, the number of people exposed to and affected by a CB attack under a given set of attack parameters (e.g., agent or dissemination method). Using casualties as the metric, the model enables assessment of overall defense system performance as different approaches are considered in each of the system elements.

In FY99, master timeline curves were generated for airborne noncommunicable (anthrax) and communicable (smallpox) biological agents and for chemical agents (sarin). These curves show quantitatively the importance of time in developing intervention strategies. An initial assessment of the relative value of protection measures and medical responses, with various levels of early detection assumed, was conducted using master timeline curves for anthrax and smallpox. The intuitive concept that complementary medical and protective strategies can be more effective than either strategy alone was confirmed by the analysis. Assessment of the candidate system and alternative defense strategies was provided in the Phase I Final Report, delivered in October 1999.
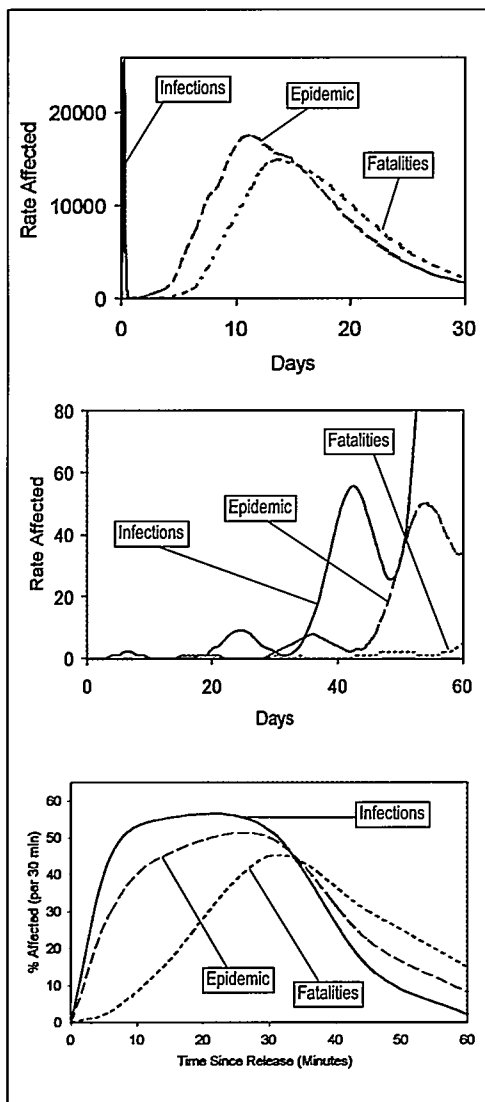
Master timeline curves for anthrax (top), a non-communicable bio-agent, smallpox (center), a communicable bio-agent, and sarin (bottom), a chemical agent. The curves illustrate the importance of time in developing effective intervention strategies.

Impact

The focus of the candidate urban defense system is to employ countermeasures to limit exposure of the population to CB agents in order to significantly reduce casualties and fatalities. The impact of this strategy is to limit potentially overwhelming demands on the medical system. The candidate defense architecture recognizes that the greatest defense leverage (casualty and fatality reduction) can be realized with combinations of defense systems

(e.g., DI&W cues protection and medical responses). Preliminary results underscore that following the release of CB agents, the major threat is time. Timely agent detection, identification, and warning are key to the effective use of countermeasures.

Ultimately, the impact of this study will be recommendations of strategies that might be adopted to implement a robust defensive system. Although the distributed organization of major stakeholders (federal, state, and local agencies and the private sector) makes centralized decision-making unlikely, the results of this study will establish a basis for evaluating the relative contributions of system components, regardless of the entity responsible. This integrated evaluation will help to realize coordinated, if not centralized, investment strategies. In addition, the study will use the framework developed thus far to conduct more extensive tradeoff studies to identify and prioritize high-value technologies. These studies will be of direct use to the CBNP in guiding its R&D portfolio.

Next Steps

Phase II of the study was initiated in October 1999 and is focusing on more detailed characterization and analysis (including system costs) of CB defense architectures. Additional master timeline curves will be developed for a broader range of agents, and further scenario-based analyses of the defense architectures and related systems will be undertaken. This work will also include a more thorough examination of the sensitivities of approaches for the defense of cities to a range of attack, operational and feasibility factors. The Phase II effort will also produce a more comprehensive survey of key technologies applicable to DI&W systems, protection measures, and medical response that could be employed, in the near term as well as the far term, to further reduce casualties resulting from CB attacks on U.S. cities.
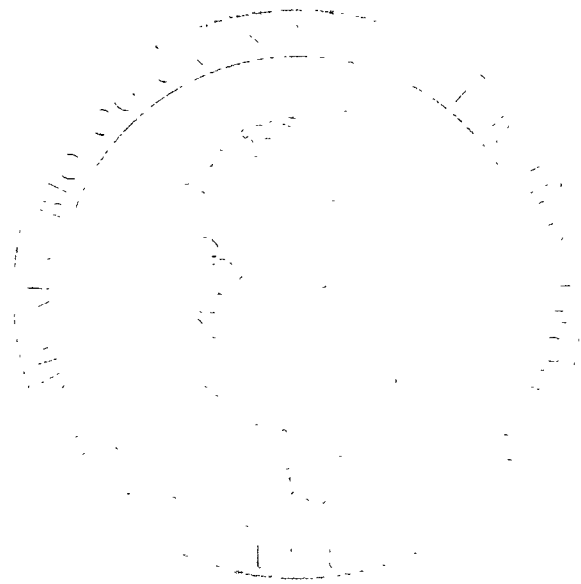
Technology Development

Technology Development is the core program element. In general, development is focused on technologies for which the basic science is already understood. The goal is to yield prototype capabilities in the three- to five-year time frame. Currently there are four areas of specific focus:

- Detection.
- Biological Foundations.
- Modeling and Prediction.
- Decontamination and Restoration.

Objectives

The DOE national laboratories are teaming in a cooperative effort to develop a modular system of high-performance sensors for chemical and biological agent detection that harness state-of-the-art science and engineering. These sensors specifically address the challenges of the mission to counter domestic terrorism and, as such, complement the DoD effort that concentrates on battlefield protection.

Although there are similarities in the desired detection systems for the two scenarios, the needs of domestic counterterrorism differ in several major respects from its battlefield counterpart:

- Counterterrorism must deal with a much broader range of agents.
- The false positive requirements are much more demanding for domestic protection.
- Detecting an attack in the vast urban population will be extremely difficult.
- There is much less supporting infrastructure in civilian populations.

Our objective is to develop an integrated detection system that meets this complex set of needs. To accomplish this goal, we are developing a small suite of portable instruments, which cover a significant portion of the 'threat space' when operated individually, but provide still greater coverage and lower false alarm rates when operated in combination.

FY99 Highlights

FY99 has seen major progress toward research prototypes in a number of areas within the CBNP detection effort. Selected highlights are described below.

Autonomous Pathogen Detection System (APDS) Lawrence Livermore National Laboratory is developing a stand-alone instrument that can provide automated continuous monitoring for many potential biological agents at special events or in high-threat locations. Building on flow cytomery

and polymerase chain reaction (PCR) techniques developed for the DOE and DoD, the focus of this project is on integrating these techniques into a fully autonomous system. The system includes continuous or on-demand aerosol sampling, sample preparation, automated fluidic sample handling and transport, detection/identification by a combination of flow cytometry immunoassay and nucleic acid recognition (PCR), and automated data analysis and reporting.

In FY99, APDS successfully met its first major milestone by operating a single flow assay autonomously and continuously for greater than 12 hours and by testing that system against a pathogen surrogate in the wind tunnel at Pacific Northwest National Laboratory.

Equally important, working with Luminex Corporation, LLNL developed a technique of using color-coded beads to simultaneously detect and identify multiple pathogens in a single flow cytometry assay.
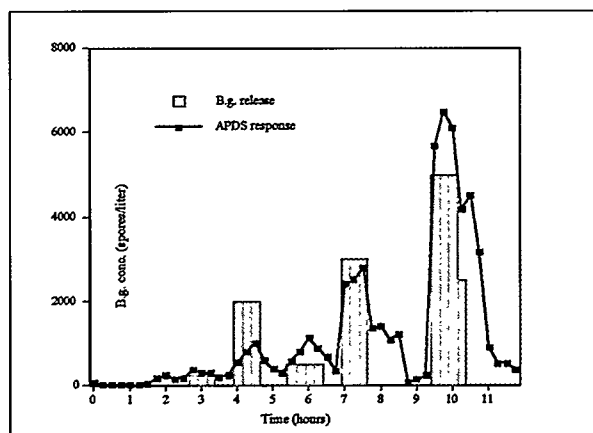
Response from the flow cytometry assay of the APDS over a 12 hour period shows very high sensitivity to challenges with a pathogen surrogate

μChemLab/CB™
Sandia and Oak Ridge National Laboratories are using an approach that is new to chemical and biological agent detection—the use of multiple chromatographic separations, each sorting on a
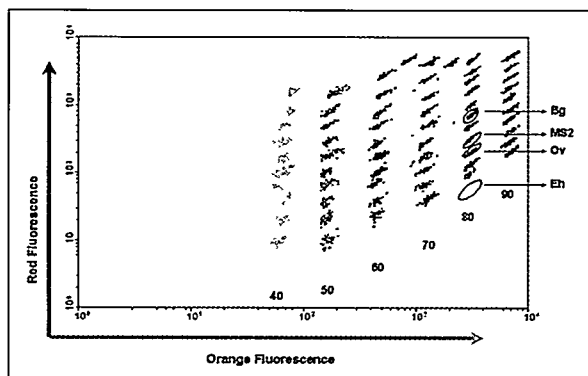
Results from a single flow cytometry assay that uses color-coded beads for simultaneous multiple pathogen detection.

Four successive magnifications of microchannels in silicon, where gas phase separation occurs.

different physical property, to provide a unique fingerprint and low false alarm rate.

Combining this methodology with microfabrication techniques, µChemLab/CB™ will be able to detect a broad range of chemical agents, biotoxins, and viral growth media signatures, all in a few minutes.

Macro view of the glass wafer containing microchannels for liquid phase separations.

In FY99, we successfully demonstrated that we can transfer this technology to chip, using etched microchannels each about the diameter of a human hair. Liquid phase separations of biotoxins were performed in microchannels etched in glass and coated so as to minimize protein sticking, with laser-induced fluorescence providing for ultrasensitive detection. We also demonstrated the gas phase detection of chemical agent simulants using a chip preconcentrator to feed a one-meter-long gas chromatograph column etched into one square centime-

ter of silicon followed by acoustic wave detection.

Advanced Ion Trap Mass Spectrometry

Under a separate program, Oak Ridge is developing a Chemical and Biological Mass Spectrometer (CBMS-II) for the U. S. Army. The CBNP effort is building on that program to develop a more capable biodetection technique that allows one to simultaneously detect and identify multiple proteins via mass spectrometry as compared with current time-consuming approaches that require first separating the biomolecules and then detecting them one at a time.

In FY99, we demonstrated a nanospray interface that allows the direct analysis of proteins, the only biomarker that is common to all three biological threats (i.e., bacteria, viruses, and toxins), and the use of ion-ion chemistry to greatly simplify the resulting mass spectra.

DNA Fragment Sizing

Los Alamos National Laboratory is developing a new method for rapid discrimination among bacterial strains by using flow cytometry to measure the length of DNA fragments. We have shown that burst sizes from intercalated dyes provide an accurate measure of the length of the DNA fragment, and that the fingerprint of fragment sizes measured by this technique allow one not only to discriminate bacterial species but also to discriminate strains within a species. Advances in speed

and sensitivity have enabled fragment distribution analyses that are about 100 times faster and 200,000 times more sensitive than the traditional method of Pulsed Gel Electrophoresis. In addition, we have greatly simplified the sample preparation protocol, reducing the sample preparation time from 18-24 hours to less than 6 hours.

Biochromic Films

Lawrence Berkeley National Laboratory is investigating polymer films that change color from blue to red upon binding a toxin, virus, or bacteria. Such films offer the promise of a very simple, low-cost detector—a type of "bioticket". The CBNP effort is focused on increasing the sensitivity and lowering the false alarm rates of these biochromic polymer-based detectors. During the past year, we gained a better understanding of the material properties that control the films' optical performance (e.g., the chemistries of the headgroups and long tails of the molecules) and synthesized a system that undergoes a reversible color change when a toxin binds.

Impact

The CBNP suite of detectors will contribute toward meeting the wide range of counterterrorism response needs, particularly first-responder requirements and enhanced warning systems for public facilities.

Firemen, law enforcement officers, and medical emergency personnel need portable, low cost, simple to use, rapid response tools to allow them to determine whether an agent is present, what it is, and its approximate concentration. µChemLab/CB™, with its ability to simultaneously address chemical, biotoxin and viral signatures in a single hand-held unit, and biotickets, in which a simple color change can indicate the presence and identity of a pathogen, are on the path to becoming two important tools in the first responders' arsenal.

Part of the nation's emerging strategy toward dealing with the terrorist threat is to monitor particularly attractive targets. To make this strategy practical, one needs autonomous, sensitive, low maintenance detection systems that can detect a broad range of agents. The Autonomous Pathogen Detection System is a major first step in this direction and uses an architecture that can subsequently incorporate other detection technologies as they come on line.

The advances made under the CBNP can also feed back into the defense community where many of these technologies have their roots. For example, fully autonomous flow cytometry and PCR can contribute both to future generations of battlefield detection systems as well as to port and base monitoring concepts now under development. The gas- and liquid-phase microseparations technologies incorporated in µChemLab™ could be an important contributor to the DoD's goal of a chemical and biological universal detector.

Next Steps

Our outyear strategy consists of three major steps:

- Bring the current programs first to a research prototype and then an engineering prototype stage.

- Take advantage of the Domestic Demonstrations and Applications Programs (DDAPs) to evaluate and refine these detection technologies in a system context.

- Undertake a small number of new starts to exploit new technologies and concepts as they emerge to fill gaps in the nation's CB defense.

The Autonomous Pathogen Detection System provides a good example of this prototype development pathway, with multiplex flow cytometry scheduled for FY00, flow-through PCR to be added in FY01, a field demonstration of the combined system in

FY02 and a commercialized prototype instrument in FY04. Similar development paths have been charted for most of the CBNP detection technologies currently under development.

Finally, we continue to look for advances in technologies that can either enhance ongoing efforts or lead to new detection approaches. Our peer-reviewed competition this past year led to two new starts for FY00.

The first, Oak Ridge National Laboratory's Advanced Multifunctional Biochip, uses a combination of bioreceptors (e.g., antibodies, DNA, and enzymes) along with on-chip fluorescence detection and a novel meso-pump technology to create a miniature, low-cost sensing platform.

The second, Los Alamos National Laboratory's Chemical Agent Detection Badge, is using a set of chemical reactions coupled with electrochemical detection to produce a lightweight (less than eight ounces) sensor that can be worn as a badge or pager.

Biological Foundations

Objective

The objective of the Biological Foundations area is to provide an integrated body of biological information and tools to support both the detection technology area of the CBNP and the needed capabilities of the nation's biological weapons defense infrastructure. This information falls into three major categories:

- Understanding the DNA of threat pathogens, which is the "blueprint" for their form and their function.

- Understanding the toxins and the virulence factors that allow these organisms to infect and harm humans.

- Developing models of how these diseases spread in a human population.

The most sensitive methods for detecting and characterizing biological pathogens are based on testing for important short segments of the pathogen's DNA. A principal objective of the Biological Foundations thrust is to characterize the DNA of potential BW pathogens and their near relatives in sufficient detail to allow detection and identification of all important biological threats and to enable forensic analysis to help determine the source of an attack.

Pathogens infect and harm humans by producing a series of virulence factors and toxins which allow them to invade the body and cause disease. Detailed knowledge of the molecular structure of these toxins and virulence factors will allow us to improve detection based on these factors and will greatly accelerate the development of vaccines and treatments.

OB–fold with GM1 sugar moiety

Ribbon model of a toxin complex.

Finally, we are also developing computational and informatics tools that will aid public health officials and researchers in recognizing an unusual disease outbreak, projecting its course in the population, and fully utilizing the wealth of data being generated by the Biological Foundations area.

FY99 Highlights

DNA Signatures
A major challenge is identifying a few small regions of DNA in a pathogen, less than 0.1% of the total genome, that will allow us to characterize the pathogen rapidly and accurately. A variety of characterization methods are needed, since some methods work better with specific pathogens and in specific situations. Having a variety of methods available also allows tradeoffs among speed, sensitivity and level of genetic resolution.

In the area of signature generation, this year we completed an extensive characterization of *Bacillus anthracis* (anthrax) strains and related *Bacillus* species. Analysis of nearly 1000 environmental samples has been used to construct detailed phylogenetic trees that allow us to rapidly identify
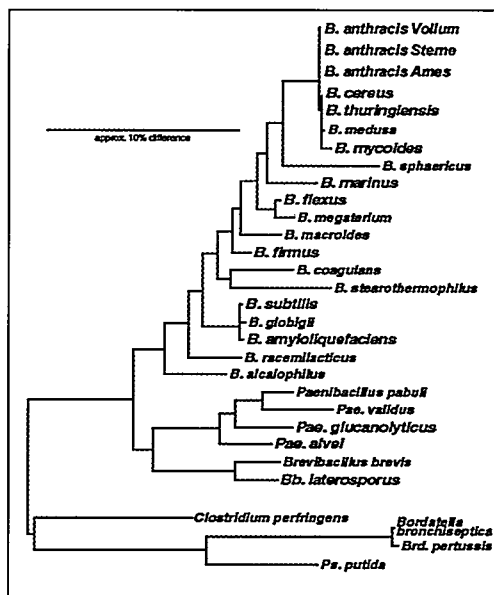
Phylogenetic tree of *Bacillus* species.

unknown samples. We have used this capability to support several government agencies in characterizing unknown or suspect samples.

We identified species- and strain-specific regions of DNA and developed PCR primers for *Yersinia pestis* (plague) and its close relatives *Y. enterocolitica*, and *Y. pseudotuberculosis*. We also developed a strain fingerprinting method for *Y. pestis*, which was successfully demonstrated using 112 strains from around the world.

Multi-locus sequence typing for species identification using species-level differences in a number of common genes was developed. Our results show that the method provides good data for establishing the phylogenetic relationships among bacteria. We applied the method to *B. anthracis* and its close relatives. The results show that *B. anthracis* is closely related to *B. cereus* and *B. thuringiensis*.

Protein Structure

A second major activity is to use information about the structure of toxins and virulence-related proteins to develop better methods of detection and aid vaccine and treatment development. Substantial progress was made this year determining the structure of the *Clostridium botulinum* neurotoxin B and *Staphylococcus aureus* enterotoxin at high degrees of resolution. Using information on toxin structures, we conducted a successful pilot study using the tetanus toxin aimed at developing artificial antibodies. Computational screening of a library of approximately 240,000 small molecules identified approximately 30 that were likely to bind to desired locations on toxin targeting
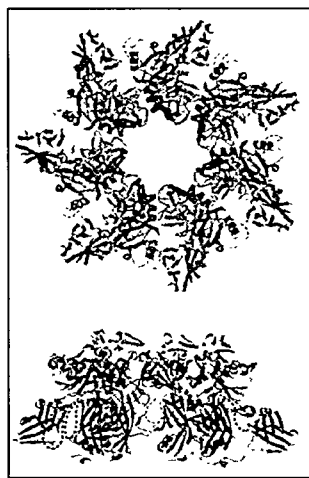
Ribbon model of the protective antigen of *B. anthracis* toxin.

domains. Approximately half of these were found to bind experimentally, and their binding strength was measured. These results will contribute to the development of prototype artificial antibodies.
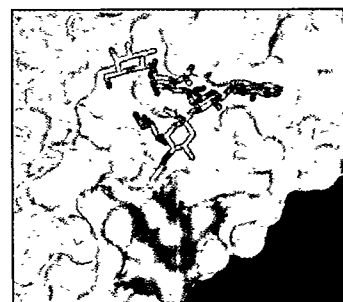
Structural model of a compound "docking" with a toxin to block harmful interactions.

Epidemiology Tools

The third primary effort is the development of epidemiology tools. Such tools are needed to recognize an unannounced attack and distinguish it from a natural disease outbreak. These tools are also needed for reconstructing an attack and predicting the course of disease spread through a population to help with crisis management.

Two pilot studies successfully demonstrated molecular recognition of "out of place" organisms using flu as a test case. Also this year, we conducted cooperative disease monitoring with medical researchers in Russia; this activity helped develop tools for tracking disease outbreaks and promoted international treaty cooperation.

Impact

The progress made to date in the Biological Foundations area is beginning to improve U.S. capabilities to detect and characterize an incident of biological terrorism, particularly as these capabilities are being transferred to other agencies. For example, our *B. anthracis* strain-typing methodology, based on variable-length repeat regions in DNA, has been transferred to the Centers for Disease Control and Prevention (CDC) for their use. In addition, our phylogenetic characterization of *B. anthracis* and its relatives is the most complete available. This information has been applied to several outbreaks of anthrax in animal populations to determine the origin of these infections.

A number of government agencies make use of our capability to analyze unknown samples. For example, we have initiated verification and validation tests which will result in our DNA signatures being transferred to the CDC's Rapid Response Laboratory (part of the CDC's Bioterrorism Preparedness and Response Unit).

Our cooperative disease monitoring pilot project with Russia has resulted in a close working relationship between U.S. and Russian medical personnel. This project found Hepatitis C to be several times more prevalent than expected in the U.S. population tested, suggesting that this serious disease may be much more widespread than previously thought. The work is also beginning to identify risk factors for the disease that will be valuable to medical personnel in controlling the disease.

Next Steps

Over the next five years, we will expand the number of agents addressed and improve the characterization capabilities available.

DNA Signatures
We expect to have species-level signatures for about 40 of the most likely BW threat pathogens and strain-level (forensic) identification for the top 10 to 15. These genetic fingerprints will be extremely specific and will have undergone extensive verification and validation. We also expect to have an initial set of species-level signatures for likely agricultural pathogens.

We have already extended signature development to six to eight new species. Strain libraries are being actively developed for *Y. pestis*, and several methods are being used and compared to construct phylogenetic relationships among the strains. A new effort has been initiated to respond to the evolving threat of genetically engineered threat agents.

A substantial effort has just been started in characterizing the backgrounds that will be present in typical samples. This work will address the problem of interference of chemical and DNA backgrounds with our assays. Recognizing the complexity of the information that will be generated by this effort, we have initiated informatics development to help with collecting, organizing, archiving, and accessing the information.

Whole-genome sequencing is under way on *B. anthracis* and *Y. pseudotuberculosis*. Having the complete *B. anthracis* genome sequence will greatly aid with detecting and defeating this pathogen, and detailed comparison of the *Y. pestis* and *Y. pseudotuberculosis* genomes will greatly improve our understanding of *Y. pestis* virulence.

Protein Structure
Over the next five years, we will also have detailed structural characterization of three to five proteins involved in the virulence pathway of each of approximately 10 pathogens for use in detection, vaccine development, and treatment options. In the coming year, we expect to complete structure determinations for all the *C. botulinum* neurotoxins and move on to other important toxins and virulence proteins in *B. anthracis*, *Y. pestis*, Staphyloccal enterotoxins, ricin, and other threat pathogens and toxins. We also expect to build and test the first prototype "artificial antibodies."

Epidemiology Tools
We will have developed epidemiology tools that will allow reconstruction of an attack or outbreak as rapidly as the raw data can be collected, and with sufficient capability for predicting disease spread to guide planning and response. This year, our epidemiology efforts are being extended to combine disease transmission modeling at the level of person-to-person contact with existing detailed models of transportation systems (air, auto, and rail). This combined capability will help us to accurately predict the spread of an infectious agent in a highly mobile society.

Objectives

Accurate prediction of the transport and dispersion of chemical and biological agents released into the environment is essential to prepare for and respond to toxic agent releases. Of particular concern is the threat to civilian populations within major urban areas where potential terrorist incidents may affect large numbers of people.

The goal of the Modeling and Prediction area is to develop an integrated and validated state-of-the-art capability for atmospheric transport and fate modeling of chemical and biological agent releases within the complex urban environment. Our approach is to adapt existing models to account for the complexity of atmospheric flows within structures (e.g., buildings and subways), around structures and over terrain. We are incorporating relevant chemical and physical behavior of gas- and particle-phase species (e.g., losses due to deposition, agent viability, and degradation) into the models and are validating the models with laboratory and field data.

Our integrated atmospheric transport and fate modeling capability is being developed to support multiple applications, including pre-incident planning, emergency response, and post-incident analysis. We are developing a suite of models with various levels of complexity and fidelity to meet the range of applications and user needs. For the complex flow around buildings and arrays of buildings, we are developing sophisticated CFD models. For the larger urban and surrounding suburban environments, we are developing algorithms to account for urban effects in regional-scale prognostic meteorology and dispersion models. We are also applying the models in a broad range of case studies and analyses to assess the vulnerability of populations in buildings, subways and open areas, to evaluate various operational procedures and response options, and to examine new methods for reducing vulnerabilities.

We will integrate the modeling tools into operational capabilities for planning and training, real-time emergency response, and post-event consequence analysis. In this regard, we are developing capabilities for authorized users to access the operational systems through the Internet. Secure access to these systems will allow users to initiate model simulations or view results of previous model simulations. Implementation of these capabilities will provide emergency managers with multi-user access to coordinate multiple-agency responses and will provide first responders with access to dispersion and prediction models in the field.

FY99 Highlights

A major effort in the past year has been to incorporate physical processes that affect transport and fate of chemical and biological agents. The airflow and aerosol physics models have been linked in the interior buildings effort. In the subway codes, we have added particle deposition due to sedimentation, forced convection, and thermophoresis as well as deposition to the tunnel and station walls.
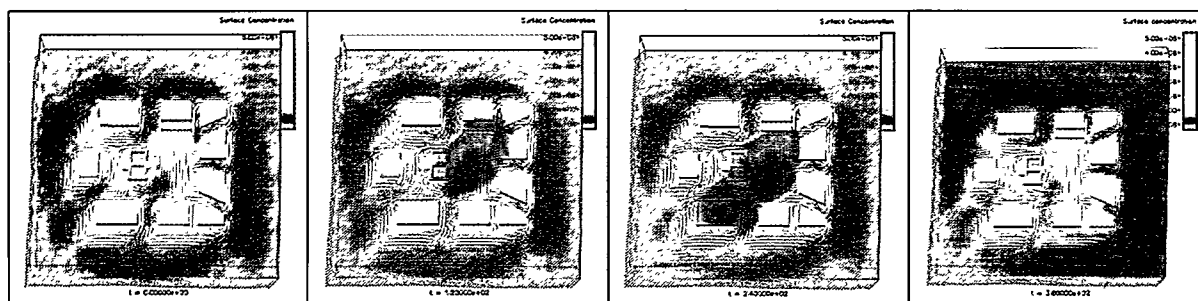
The evolution of the threat from a biological release.

A critical addition to the subway code has been the transport of agent due to uptake and discharge from the train cars. For flows around buildings or building complexes we have added surface energy budget and vegetative canopy effects to the models that simulate the complex flows. The effects of buildings and urban areas have been included in the regional-scale models by developing a scheme that senses the effects of the buildings and urban surfaces without explicitly resolving them. This was done by modifying the physics for radiative trans-
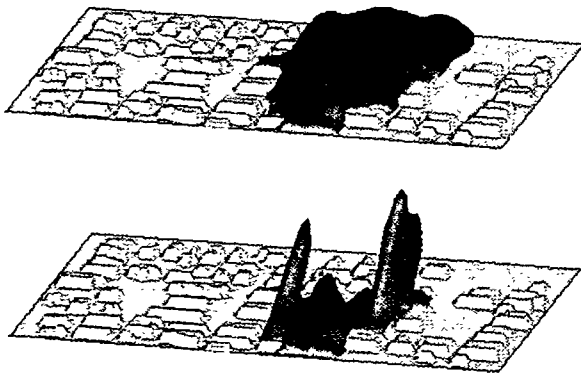
The calculated dispersion of a threat agent without accounting for radiation transfer (top) differs significantly from the same calculation when radiation transfer is included (bottom).

fer, the surface energy budget, and turbulence production and including anthropogenic heating and rooftop energy balance.

The models are being tested and validated using data from previous laboratory and field experiments and by conducting new experiments. A preliminary outdoor release experiment was conducted in FY99 to examine penetration of micrometer-size particles into a test building. The subway models are being compared to data from the 1966 biosimulant release in the New York subway system and to data from experiments conducted in the PROTECT program. The models for simulating the complex flow and dispersion around the exterior of buildings are being compared to data from laboratory wind-tunnel experiments and

field data. Regional-scale models are being validated using routinely available meteorological data, and we are awaiting data from CBNP field experiments for further validation.

As an example of the modeling work, a simulation using the subway model indicates that, after some time, a major fraction of a bioaerosol released inside a subway system is vented to the outside. The residual agent creates a decontamination problem within the subway system, but the vented portion indicates the need to couple the subway model to both local- and regional-scale exterior models. Simulations with the building-scale models have shown the effects of neighboring buildings as well as the interaction with the vegetative canopy. Regional-scale simulations have demonstrated the effect of the urban heat island, where increased turbulence increases agent dispersion and plume spread.

A biological release within the subway system vents a large fraction of hazardous material to the outside.

A prototype version of the Internet remote access system has been completed. This software provides the ability to enter basic information about an atmospheric release of selected chemical or biological material, communicate with the models within the National Atmospheric Release Advisory Center (NARAC), and view basic maps of predicted health hazard zones. The graphical user interfaces provide a look and feel that will be familiar to most personal computer users, reducing the initial learning curve. The basic graphical display tool allows viewing and archiving of model predictions along with maps that are pre-loaded on the remote

user's computer. This year, we demonstrated the ability to complete a "round-trip", from event scenario input to model product delivery, using the remote access prototype system.

Impact

The modeling and prediction capabilities being developed are unique and will provide users in the intelligence, law enforcement, and emergency management communities with a heretofore unrealized ability to better protect U.S. citizens. With the development of documented and validated models of the transport of material within urban settings, and with improved detection and communication of results to users, we will greatly improve our capability for incident response.

The models being developed will have applications in pre-planning and training, emergency response, and post-incident analysis. For example, pre-planning analyses are essential to determine the locations of emergency assets and egress routes. During an incident, modeling predictions are needed to predict the downwind hazard, and to determine safe-zones. After an incident, modeling can be used for event reconstruction and to aid in identification of those exposed.

Next Steps

In the coming year, we will pursue a variety of activities to advance the development of an integrated system for modeling atmospheric transport and fate that is both scientifically state-of-the-art and responsive to emergency management and first responder needs. We are continuing model development in all areas to improve urban physics, the treatment of CB agent-specific behavior, computational efficiency, and user interfaces. Model validation will be an area of particular emphasis and will focus on exterior flows around buildings and over larger regions. As part of this effort, we are planning for field experiments and model comparison studies.

Objectives

The objective of the Decontamination and Restoration area is to develop rapid, effective, and safe (nontoxic and noncorrosive) decontamination technologies for the restoration of civilian facilities following a chemical or biological attack. Within this area, we address the decontamination of open (stadiums), semi-enclosed (subways), and enclosed facilities (buildings) as well as sensitive equipment. Decontamination will be performed by multiple users, presenting technology development challenges. For example, for the first responder, it is critical that facilities or equipment be decontaminated in a very short time so that any casualties can be located and treated. During the restoration process, time is of less importance but collateral damage, public perception, and re-certification are of greater consequence. Finally, there are social, political and regulatory issues that must be considered.

FY99 Highlights

Work in FY99 focused on the development of several decontamination technologies as well as the development of a strategy for understanding the likely regulatory compliance issues. Selected accomplishments are summarized below. More detailed information is given in the Appendix.

DF-100 Aqueous Foam

A nontoxic, noncorrosive aqueous foam with enhanced physical stability for the rapid mitigation and decontamination of CB agents has been developed at Sandia National Laboratories. Results to date have shown effective decontamination of both CW and BW agents. The chemical agents GD, VX and HD are completely neutralized after exposure to the foam for several minutes. In addition, 99.99999% kill of *B. anthracis* spores was achieved after a one-hour exposure to the foam solution. Tests have also demonstrated that the foam is effective in killing vegetative cells of *Erwinia herbicola* and the bacterial virus MS2, which are used as simulants for bacteria and viruses respectively.

The foam has been successfully deployed by small fire extinguisher-type units (pressurized by CO_2 cartridges), by hand-held units that are pressurized by a connection to a fire hydrant, and by large military-style pumps. DF-100 was demonstrated in the Fixed Site Decontamination Trials at the Edgewood Chemical and Biological Center, where the foam successfully neutralized TGD (thickened soman), VX and HD.

Decontamination foam safely neutralizes chemical and biological warfare agents.

L-Gel

Lawrence Livermore National Laboratory has evaluated various oxidizer systems as reagents for detoxification and/or degradation of CB agents to nontoxic environmentally acceptable components. Gels have the advantage of adhering to vertical surfaces and even the underside of horizontal surfaces such
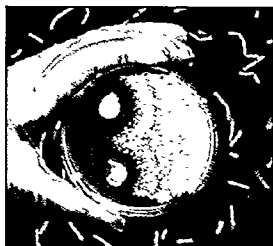

The Decontamination and Restoration effort seeks decontamination technologies for the restoration of civilian facilities—open, semi-enclosed and enclosed.

as ceilings and walls, thereby maximizing the contact time. The primary gel decontamination system now under development relies on an amorphous fumed-silica gelling agent combined with the commercial oxidizer oxone manufactured by DuPont. Experimental testing on both surrogates and live chemical agents has shown the L-Gel system to be effective for complete CW decontamination and for all biological surrogates/spores as well as live vaccine strains (*B. anthracis Sterne*). The final formulation is relatively noncorrosive (pH approximately equal to vinegar), and EPA testing on the residual materials from surrogate experiments has shown residues to be nonhazardous.

L-gel inhibits germination of *B. globigii* spores (the brown region around the two light circles). The right side of the dish shows viable cultures of *B. globigii.*
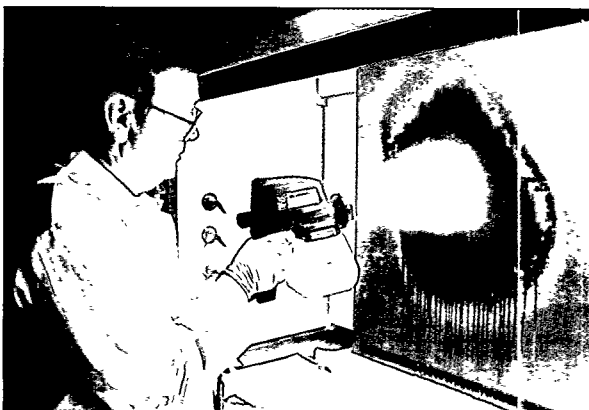
Application of L-Gel through a commercially available paint sprayer

Gases

While liquids, gels and foam-based reagents may be effective in decontaminating exposed surfaces, reactive gases may also be necessary to complete a full decontamination, since gases are the only practical means of getting into small cracks, cul-de-sacs, microporous materials and air ducts. Ozone gas was investigated as a baseline, and a number of practical issues that may affect the use of gases have been identified.

Under the right environmental conditions, ozone can be an effective reagent against biological agent surrogates in as little as one-half hour. However, due to the difference in density between gases and liquids, the rate of destruction of chemical agent surrogates is considerably slower. Reactive gases other than ozone (for example, chlorine dioxide and vaporous hydrogen peroxide) are now being examined for their ability to decontaminate CB agents.

Plasma Jet

Atmospheric Pressure Plasma (APP) technology for CB decontamination is under development at Los Alamos National Laboratory. The goal is to convert a mix of innocuous gases, such as helium and oxygen, into a reactive gas stream capable of detoxifying CB agents. APP decontamination devices may provide a much needed method of CB decontamination which, unlike traditional methods, is dry and nondestructive to sensitive equipment, like electronics, and irreplaceable objects, like national treasures. APP could provide a fast and portable means of restoring contaminated items for which the only current option is disposal.

In recent tests, the reactive effluent of the APP jet has been shown to kill *Bacillus globigii* (BG) spores, a surrogate for anthrax, with a D value (time to reduce viability by a factor of 10) of 4.5 seconds at an exposure temperature of 175°C and a stand-off distance of 0.5 cm. This is 10 times faster than hot gas at the same temperature and requires approximately 80% less energy to achieve the same level of kill. The APP jet has also been shown to neutralize surrogate and actual CW agents including mustard and VX.

Research efforts are now being directed toward reducing helium consumption and increasing the allowable stand-off distance for effective decontamination. Alternative feed gas compositions have shown great promise in these efforts.
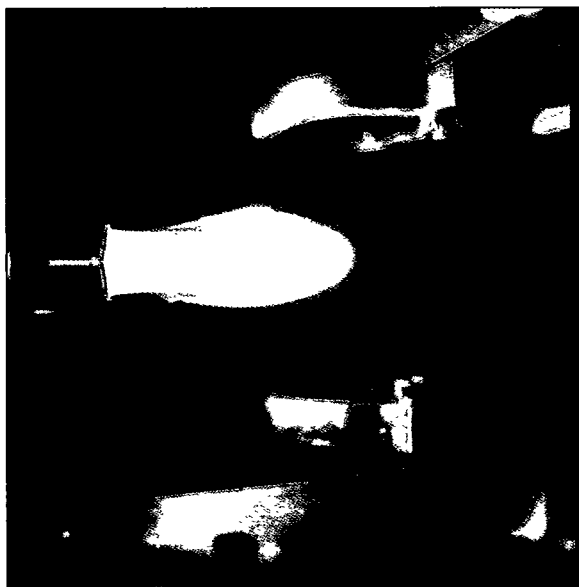
The low-temperature atmospheric pressure plasma jet.

How Clean is Clean Enough?

The overall issue of How Clean is Clean Enough? and the methods by which this is determined (e.g., sampling and verification) are key to establishing effective and successful decontamination methods. The primary goal of this effort is to determine the level of cleanup that will be required to meet both regulatory and stakeholder needs.

Over the last year, a summary of available dose values and exposure limits for specific CB agents has been compiled, primarily based on military data. A review of existing environmental regulatory limits has also been completed. These data are being used to estimate the likely cleanup levels required following a CW attack. For BW cleanup, the issues are more complicated since many organisms are indigenous, and risk is a function of effective dispersion, exposure (i.e., inhalation) and infectivity. We are working to adapt existing guidelines.

As part of this effort, we have developed a conceptual Biodecontamination Decision Process to help first responders and emergency response managers determine appropriate actions following a terrorist attack. This decision framework highlights a series of decision points, including initial notification,

first responder response, restoration of operations, and longer-term remediation.

Impact

New decontamination and restoration technologies are one important element in responding to potential CB attacks. Technologies developed as a result of this effort will allow the rapid, safe and complete restoration of domestic facilities in the event of such an attack. These technologies will allow reuse of a contaminated facility in a timely manner without the loss of critical and expensive equipment.

Next Steps

Work in the coming year will focus on several key areas.

- Development and optimization of the decontamination technologies described above will continue. Live agent testing will be a particular focus for the more well-developed technologies.

- Work will focus on the development of equipment and techniques for field deployment of the decontamination technologies. These efforts will involve the selection of equipment, such as foam generators, to allow the decontamination formulations to be rapidly and efficiently deployed.

- We will be participating in a number of field and demonstration exercises aimed at moving complete decontamination systems into the field.
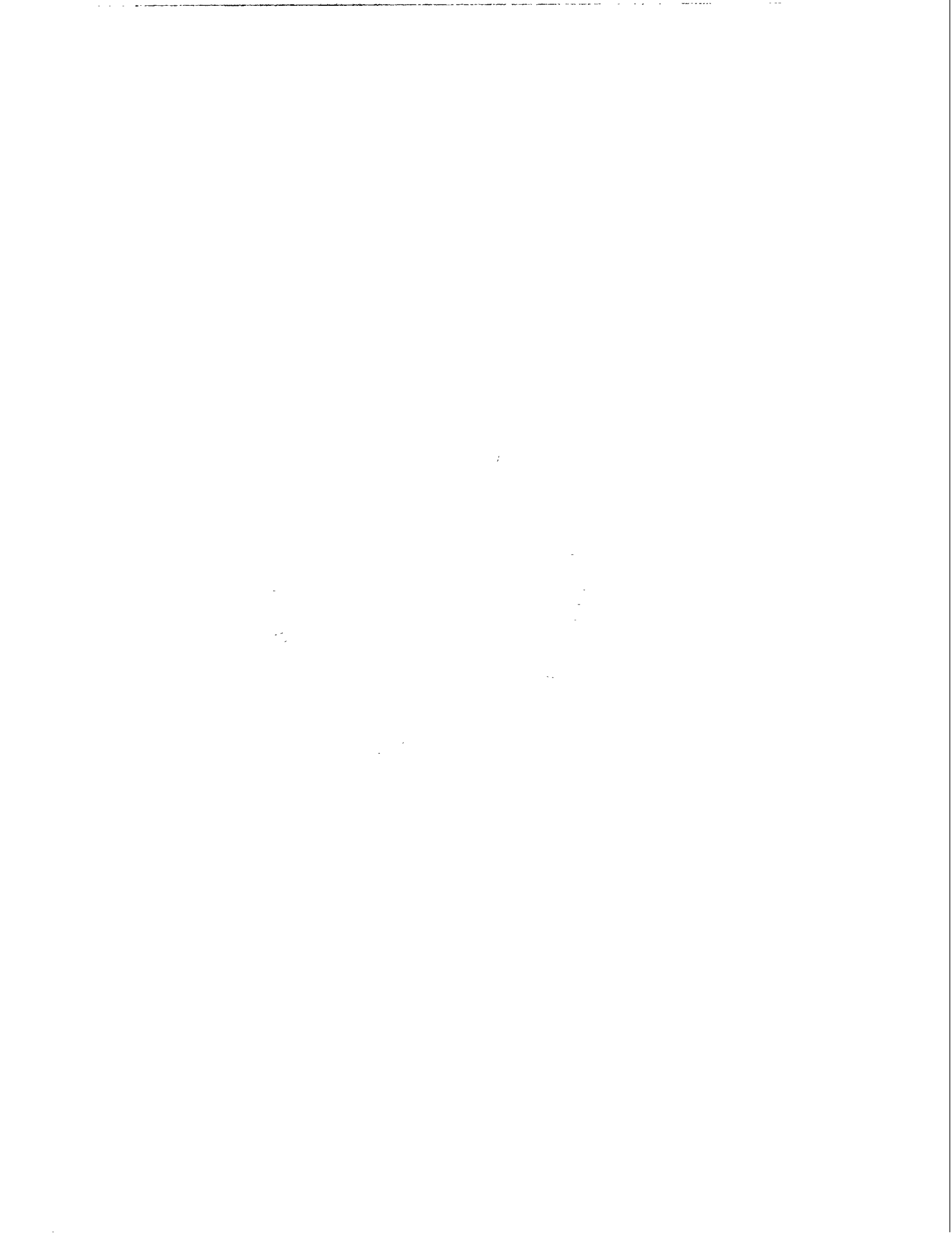
Domestic Demonstration and Application Programs

Domestic Demonstration and Application Programs (DDAPs) are focused on demonstrating the potential impact of technology, integrated into a system, to address specific problems facing a CB Urban Defense system. The two current DDAPs are focused on developing and deploying detection, identification and warning systems.

The PROTECT DDAP is targeting vulnerable facilities characterized by high concentrations of people. Subway systems have been the initial focus, with airports as a secondary emphasis. The initial subway demonstration will feature in-station chemical detectors linked to an operational control center. This will provide the potential for real-time detectors to cue such operational responses as controlling trains and exhaust fans, or moving people out of hazard zones promptly.

The BASIS DDAP is designed for limited duration monitoring for airborne biological agents during a special event or period of heightened alert. Examples of special events include major sporting events, political conventions and international summits. The BASIS system is capable of wide area outdoor coverage and will enable prompt screening for exposure and subsequent medical treatment by appropriate health authorities.

It is expected that each DDAP will be conducted over 2–3 years, with follow-on demonstrations as appropriate. In addition, new DDAPs will be initiated as opportunities for implementing technology based systems are identified.

PROTECT: CB Defense of Infrastructure

Objectives

The Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism (PROTECT) integrates technologies, including those developed under the CBNP, into a unified approach for protecting fixed infrastructure. The PROTECT process for each infrastructure covers site characterization, response options evaluation, implementation, and maintenance (see the PROTECT Process figure below). The CBNP areas of detection, modeling, and decontamination play important roles in the PROTECT process. Demonstrations of the integrated technologies are planned in at-risk facilities, including subways, airports, high-threat buildings, and interior structures at special events.

Steps	Phase
Conduct Threat Assessment	Define Problem
Perform Vulnerability Analysis	
Develop Response Strategies	Investigate Options
Design System Upgrades	Implement Solution
Install and Test Upgrades	
Conduct Training	Maintain Readiness
Maintain and Update System	
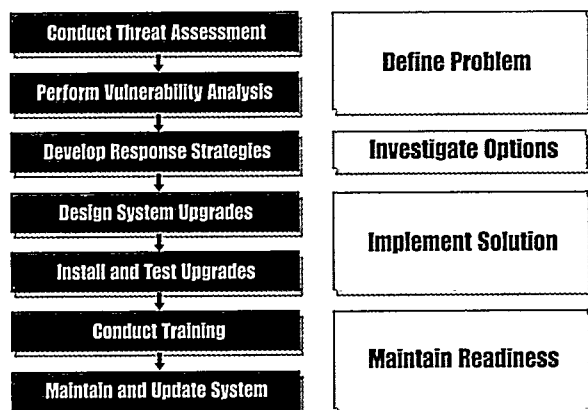
Steps in the PROTECT process for high-threat infrastructure protection.

FY99 Highlights

Subway program
The PROTECT subway program is the most mature component of the infrastructure protection effort. The principal collaborator for this program is the Washington, DC subway (WMATA).

Incident Response
Interim response guidelines have been developed with WMATA personnel based in part on modeling of alternative response strategies using models developed under the CBNP.

Detection and Warning
A subway station has been chosen as the site of a chemical agent detector test-bed. The test-bed will be used to evaluate the operation of detectors over long periods of time for false alarms and operational problems in a dusty environment. Smoke tests done in that station in September 1999 provided a visual and quantitative measure of how chemical agents would behave if released in a station, both with moving trains and with no trains operating. Sixteen smoke releases were carried out to better characterize airflow when trains enter and leave the station. This characterization provides more precise guidance for detector placement and emergency response. We anticipate that the chemical detector test-bed will be in operation with detectors installed by the spring of 2000.

We conducted an evaluation of commercial chemical agent detectors, and three detectors have been selected for testing and evaluation in the subway. The sensor and warning program, including detectors, gas-sampling manifolds, alarm buttons and video systems, has been considered in the context of various scenarios. The objective here is rapid determination that an incident is occurring—every minute of faster response can save tens to hundreds of lives depending on the exact circumstances.

Decontamination
Special requirements for decontamination and restoration of service after a chemical incident have been developed based on the discussions with the Washington, DC and Boston subway staffs. Contacts with the National Medical Response Teams (NMRTs) have led to discussions of subway needs versus NMRT chemical agent decontamination capabilities. This information is also being coordinated with DOE decontamination researchers.

Airport program
Airport terminals are representative of an important class of large, air-conditioned interior facilities and are themselves vital infrastructure elements.

Initial work to guide the development of effective responses to the CB threat to airports has begun in the PROTECT program. These responses will use the modeling and detection capabilities developed under the CBNP. A collaborative agreement with an airport has been reached that focuses initially on determining the airflow in large terminal facilities. As with the subway interaction, support of broader emergency operations planning has been initiated within the airport partnership.

Impact

Large high-threat interior structures are a source of concern for CB protection in the U.S. These include subways, airports, and government office buildings, where people are concentrated in small areas, and quick evacuation is difficult. Such structures may be terrorist targets, and protecting them is an important goal. Much can be done to reduce the impacts of an attack on fixed facilities. Use of detection, modeling, and decontamination technologies for one type of infrastructure can be extended to other types without major modification in the methodology. The methodology shown in the PROTECT process figure applies to all types of interior structures, since the basics of response are similar.

Application of the technologies to the variety of interior infrastructures requires partnerships with the site managers. DOE technology is best applied with collaboration of the user community. Once representatives of each type of interior structure have been protected, the technologies used will be transferred to the wider user community and to industry.

Next Steps

A major activity will be the subway station detector test-bed study, with possible inclusion of a prototype DOE μChemLab/CB™ chemical detector. Development of alternative warning technologies, such as pattern recognition systems, will be pursued. A prototype of the responder tool called CB-EMIS will be developed to facilitate easy video, graphics

and text exchange of information among first responders, the Incident Commander and the Operations Control Center for a subway incident. The planned response tool is illustrated in the figure below.
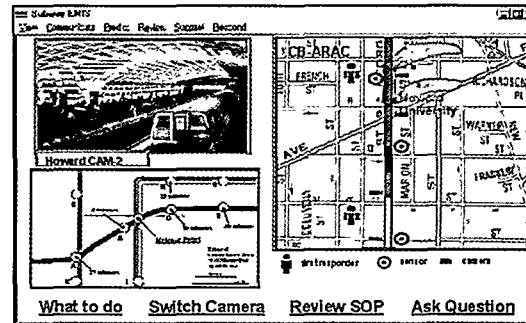
Sketch of the CB-EMIS tool for crisis management to be used for a large high-threat interior infrastructures.

The airport program will develop a comprehensive process and supporting technologies to enable airport operators to develop effective operational response plans. It will guide their decisions regarding investments in detection and mitigation architectures. The initial activities at the airport will include testing and analysis to characterize the air flow within the terminal. This information will permit immediate planning for heating, ventilation and air conditioning (HVAC) response measures that can minimize the impact of a chemical release. It will also provide the basis for development of sensing and warning architectures.

The chemical agent test-bed results and analysis methodologies developed in conjunction with the subway project should be applicable to airport systems. The extensive video surveillance assets already installed at airports will be an important component of the near term CB sensing and warning system. The airport partnership will work toward an integrated demonstration of linked detection and control systems.

The methods developed for the subway and airport work will be extended to high-threat office buildings and to interior structures housing special events.

BASIS: Defense Against Bioterrorism at Special Events

Objectives

The goal of the Biological Aerosol Sentry and Information System (BASIS) is to develop and demonstrate a biological early warning system to provide civilian medical and law-enforcement authorities with timely and accurate information about biological aerosol attacks. One of the key objectives of BASIS is to reduce greatly the time needed for reliable detection and identification of a biological aerosol attack, so that medical treatment can begin as soon as possible, thereby limiting the number of casualties. The initial application of BASIS is to complement security operations at special events, but the system will also be useful in other situations such as when intelligence or other warning indications are available. The BASIS program is structured to demonstrate the biological early warning system in early FY01. The completed system will then be made available for a special event in early FY02.

FY99 Highlights

The BASIS program was initiated in FY99, with primary tasks focused on user interactions, definition of the overall architecture and prototype tool development. In FY00, the program is concentrating on preparations for a major field demonstration, scheduled for early FY01.

In the last year, important groundwork was laid for the BASIS program. Discussions with officials in the Public Health Service, the Centers for Disease Control and Prevention, the State of Utah, and Los Angeles County have led to the development of the system requirements. Other agencies, such as local law enforcement, fire and emergency medical services, and Federal agencies such as the FBI and Secret Service, have also provided valuable input to the system definition. Through these interactions, we have developed the architecture for a biological early warning system. This architecture emphasizes sensitive, wide-area coverage that is constrained by the state of science and technology and by the available funding for the program. The BASIS architecture consists of four major elements along with decision support tools.

Distributed Sampling Units (DSUs)

DSUs monitor aerosols and collect aerosol samples for analysis. By using many such units, wide-area coverage is provided at a modest cost. An additional attribute is flexibility; samplers can be easily and quickly relocated to other sites.

Relocatable Field Laboratory (RFL)

The RFL provides high-throughput analysis of aerosol samples using very sensitive techniques with high specificity for selected biological agents. On-demand testing is available for a wide range of biological agents. The aerosol sample archive is also maintained in the RFL to provide a comprehensive history of aerosol at monitoring sites for *ex post facto* analysis. The use of the highest quality assays in the dedicated laboratory ensures that false alarms will be minimized.

Command Console (ComCon)

The ComCon provides a capability for real-time monitoring of aerosol information from
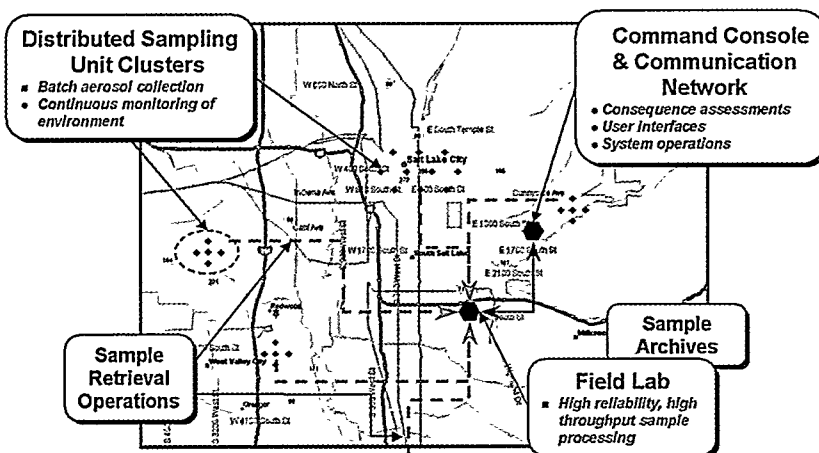
The BASIS architecture.

the DSUs and analysis results as they become available in the RFL. The ComCon's decision aids and planning and training tools provide public health and other officials with information to incorporate into their response to a biological attack. For example, following a positive analysis, prediction of hazard zones and safe areas may be initiated immediately.

Communication System

The communication system provides multiple, robust communication paths among the DSUs, the RFL and the ComCon.

Decision Support Tools

As part of the BASIS architecture, we have initiated development of a suite of decision support tools. These tools include the Virtual Planner, which allows users to easily simulate the effects of a release of a chemical or biological agent and model the sensor response of various exposure levels, including estimates of populations affected at different levels (threshold reactions, incapacitation, various degrees of lethality). A web-browser-based tool for rapid access to reference information, operational check-lists, and detailed facility information (known as "target folders") have also been developed.

The BASIS decision support tools were deployed during the Westwind WMD exercise in Los Angeles in February 1999, in support of the Los Angeles County Operational Area Terrorism Early Warning (TEW) group in the Los Angeles County Emergency Operations Center (LACEOC). Analysis results and data from the BASIS tools were used extensively in the LACEOC initially and then quickly provided to the field command post and Joint Operations Center (JOC). This exercise served as an early (and successful) demonstration of the BASIS decision support tools.

BASIS information was prominently displayed in the Westwind Joint Operations Center.

Impact

One of the key factors in saving lives following a biological attack is time. The sooner medical treatment can begin, the more lives that can be saved. BASIS will allow early initiation of medical response to a covert biological agent attack. Most importantly, BASIS offers the opportunity for a completely new approach in civilian public health response to biological agent attacks. It does this by providing an architectural foundation that allows the incorporation of advanced biological detection and identification technologies as they become available. BASIS also allows for the extension of monitoring for biological agent attacks to larger areas for extended periods of time.

Next Steps

BASIS will move forward with development, validation, integration, and testing activities. Key activities include an extensive validation program for the field analysis protocols and a DSU performance demonstration test series in FY00. In FY01, a system prototype integration test will be executed, to be followed by the DDAP Demonstration in February 2001. Based on successful completion of the demonstration, the early warning system will be made available to an identified special event.

Other important Federal activities are underway to improve our preparedness and response to the potential use of chemical or biological agents. The Department of Health and Human Services (HHS) has formed National Medical Response Teams, is conducting research and development, and is stockpiling new vaccines and therapeutics. The Department of Defense plays a key role through its Joint Task Force for Civil Support. Response teams have been formed and training of responders in cities around the country is underway. New technologies that may have application for the warfighter and for domestic use are being developed. The interagency Technical Support Working Group supports a range of counterterrorism technology development efforts, targeting incremental enhancements that can be implemented in a 12 to 18 month time frame. The Department of Justice, through the Federal Bureau of Investigation and the National Institutes of Justice, plays a key role in the development and use of technologies to aid in criminal prosecution. Finally, the National Domestic Preparedness Office has been formed to aid in coordination and communication with state and local officials involved in domestic preparedness.

The DOE CBNP is designed to complement these and other programs. To avoid duplication of effort, the CBNP interacts with related efforts by a number of formal and informal coordination mechanisms. Formal coordination occurs via the Counterproliferation Program Review Committee (CPRC), the Nonproliferation and Arms Control Technology Working Groups, and the NSC-led Weapons of Mass Destruction Preparedness, Consequence Management, and Protection Group (WMDP). An important activity in the last year was the formation of a Chemical and Biological Defense Focus Group under the CPRC. This group will lead the integration of the DOE and DoD CB R&D activities through the development of joint technology development roadmaps.

Informal coordination occurs routinely via information exchanges and working-level contacts between the CBNP and others engaged in combating chemical and biological terrorism. The CBNP also sponsors an annual meeting to review the status of the program, and attracts participants from across the chemical and biological defense and counterterrorism communities.

Where appropriate the CBNP jointly funds projects with other agencies with a common interest. For example, in FY99 joint projects were funded to develop and evaluate systems concepts for defending cities from chemical or biological attack, and to sequence the genome of *Bacillus anthracis* to identify the portions responsible for virulence and to develop better signatures for detection.

Budget Summary

The CBNP was established with an initial FY97 budget of $17 million and has been supported at $18.5 million in each of FY98 and FY99. The FY00 CBNP budget is $40 million. Shown below are the budget allocations to the major components of the CBNP in FY99 and FY00. Beginning in FY00, the program is expected to experience a series of budget increases to enable the more rapid development of needed capabilities. These funding increases will support the development and delivery of equipment prototypes to end users, the PROTECT and BASIS system demonstrations, and planning and definition of new demonstrations.

FY99 Total Budget $18.5 M

- Other $1.5 M
- Studies $0.2 M
- DDAPs $1.6 M
- Detection $7.3 M
- Modeling $2.0 M
- Decontamination $1.6 M
- Biofoundations $4.3 M

FY00 Total Budget $40.0 M
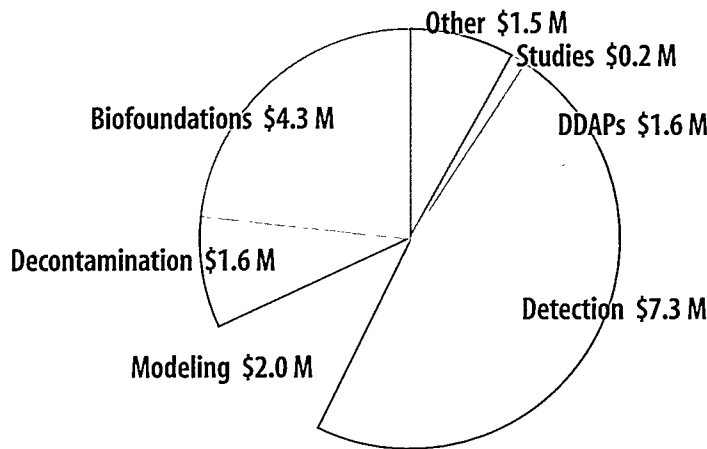
- Other $3.7 M
- Studies $0.5 M
- DDAPs $7.0 M
- Detection $11.3 M
- Modeling $5.0 M
- Decontamination $2.2 M
- Biofoundations $10.3 M
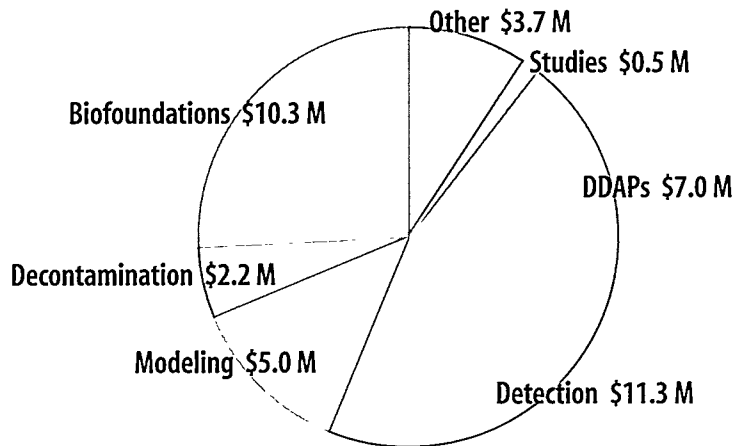
Looking Ahead

Progress in FY99, described in this report, has set the stage for program acceleration in selected areas. Examples of key anticipated accomplishments are shown in the CBNP five-year "roadmap" below. The linkages between the technology development efforts and the demonstration programs are also indicated.

In FY00, important progress will be made in many areas. Two areas, detection and demonstrations, deserve special attention. There will be a major demonstration of the phase I prototype of the μChemLab/CB™, the next generation of chemical and biological toxin detection based on microseparations technology. In addition, we will be fabricating six hand-held biological detectors based on PCR technology and providing these instruments to state and local responders in an operational "beta" test. We will also aggressively move forward with our PROTECT and BASIS programs. The chemical detector testbed will be emplaced in a major metropolitan subway system, and all elements will be readied for an early FY01 demonstration of the BASIS biological warning system for special events.

CBNP Roadmap: Major Milestones and Deliverables

		FY00	FY01	FY02	FY03	FY04
DDAPs	PROTECT	Subway vulnerability assessment/response strategies ○	Integrated chemical detection demonstration (one station) ○□	Chemical detection system demonstration at five subway stations ○□	Emergency mgmt and training/bio detection demonstration ○□▲	Preparedness system fielded for four subway systems ○□◇▲
	BASIS	Architecture development for city protection ○	Integrated biological early warning system demonstrated for metropolitan area ○	Expanded/ruggedized bio detection system for use in a metropolitan area ○□	Architecture development for 2nd generation systems ○□	Enhanced system with deployment to other cities ○□▲
	Forensics		Bioagent "geolocation" capability for two pathogens □▲	Limited capability to recognize genetically engineered agents □▲	Technological protocols for event reconstruction ○▲	"Geolocation" and engineered agent ID for additional agents □▲
	Decontamination			System design studies ◇	Mobile gel and foam systems demonstrations ○□◇▲	Initial system fielded with sampling & analysis tools ○□◇▲
Technology Development	Biological Foundations ▲	DNA fingerprinting of top five BW pathogens	Laboratory standards for genetic analysis using DNA fingerprints	Identification of virulence pathways for five BW agents	Ten-fold improvements in time and cost for DNA based detection	Structure/function relationships determined for top ten biotoxins
	Modeling and Prediction ○	Guidance for response to releases in office buildings	Validated model for flow prediction in interiors/subways. Web access to CB models for planning	Integrated int/ext. model for vulnerability analysis	Begin transition to operational capability	Operational outdoor predictive capability fielded for national use
	Detection □	Hand-held prototype tested on top chem & biotoxin agents	Ten-fold increase in sensitivity of personal "bioticket"	Field test prototype hand-held chemical and biotoxin detector	Field test autonomous sensor for ten BW pathogens	Field test virus module in hand-held chem & biotoxin sensor
	Decontamination ◇	Live agent testing with environmentally benign gels & foams	Gel and foam systems fielded		Dry plasma-based system tested on broad range of materials	

▲ ○ □ ◇ Icons show how technology development initiatives feed into DDAPs.

We will continue to work closely with our interagency partners to ensure that information is shared, and work is not duplicative. We will continue to participate in the WMDP R&D subgroup to develop the government-wide R&D plan to counter the threat of use of weapons of mass destruction, and will contribute to government-led training exercises as a way of bringing our capabilities out of the laboratories and into the field. Finally, we are forming key international partnerships, and have begun to explore the value of having the states of the former Soviet Union contribute to selected technological areas.

The CBNP will face a number of programmatic issues in the coming year. Our budget has more than doubled from FY99 to FY00, and we must work to ensure that we have appropriate staff at DOE and the national laboratories to maintain the program's high quality during this period of growth. The reorganization of the DOE and the formation of the National Nuclear Security Administration may affect the program, and we will work to ensure that the CBNP maintains its focus and is able to make use of all of the DOE laboratories. We will continue to rely on technical peer review to aid in the selection of the highest quality, highest impact projects. Finally, we will actively solicit the participation of academic institutions and industry to complement the role of the DOE laboratories.

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Appendix: Project Summaries
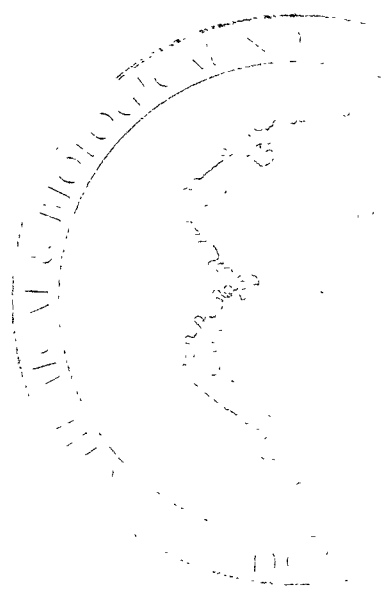
Defense of Cities Against Chemical and Biological (CB) Weapons Attack Study – Master Timeline Curve Model

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Objectives

The concept of *master timeline curves* was developed as an analytical means for understanding the cause-and-effect relationships during a CB attack on civilian targets (see figures below for notional BW contagious and noncontagious agents). The curves integrate the human consequences of the three phases that characterize a CB attack:

- The Infection Contamination Phase (the release, dispersion and subsequent exposure to agent and subsequent probability of infection/contamination)
- The Symptoms Phase (course of physiological effects and clinical manifestation from being infected/contaminated by agent)
- The Outcome Phase (surviving the exposure, debilitation or death of the infected causalities)

A master timeline curve depicts the time evolution of the affected population for each of these phases. Collectively, the set of master timeline curves describes the temporal relationship between these phases and provides a means to visualize the sequence of events that characterize the CB attack. At this level they provide an analytical tool for characterizing attack scenarios and assessing the functions and requirements of a defensive architecture. Taken individually, the curves provide a framework for developing a deeper analytical knowledge of the underlying parameters that control the net effects within each phase.

In the Defense of Cities study the goal was to develop an assessment tool that would allow a comprehensive perspective that the *defender* could use in addressing the prime objective of saving lives. The master timeline curve creates this macro-perspective while allowing for a hierarchy of increasing levels of detail. The use of the curves has proven insightful both in understanding the chain of effect and consequence for a given attack scenario and in performing sensitivity analysis and parametric assessment of potential defensive strategies, technologies and system counter-measures. Any future implementation of defensive architectures and strategies, passive or active, requires this breadth of understanding.
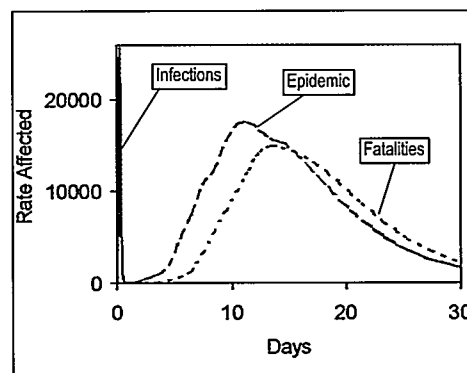
This timeline curve depicts results for an outdoor point release of noncontagious BW agent (e.g., anthrax) in which no defensive response takes place.
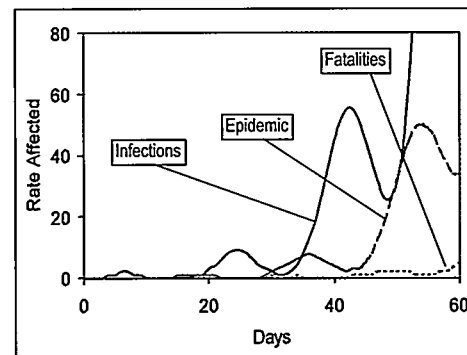
This timeline curve depicts results for an indoor point release of contagious BW agent (e.g., smallpox) in which no defensive response takes place. The curve illustrates the relatively long period for the agent and the second generation of infection resulting from human-to-human transmission.

Constructing the Master Timeline Curves

The Infection Curve

The infection curve represents the time rate at which the population can be expected to be infected or poisoned as a result of the release, dispersion, and population exposure to the agent (see figure below). In algorithm terms, it is the cross correlation of the agent release characteristics (e.g., location, aerosolization mechanics, agent) and the dispersion phenomenology (e.g., local meteorological, terrain or other environmental conditions or movement of agent carrying vectors) in the same environment space occupied by a dynamic human population.
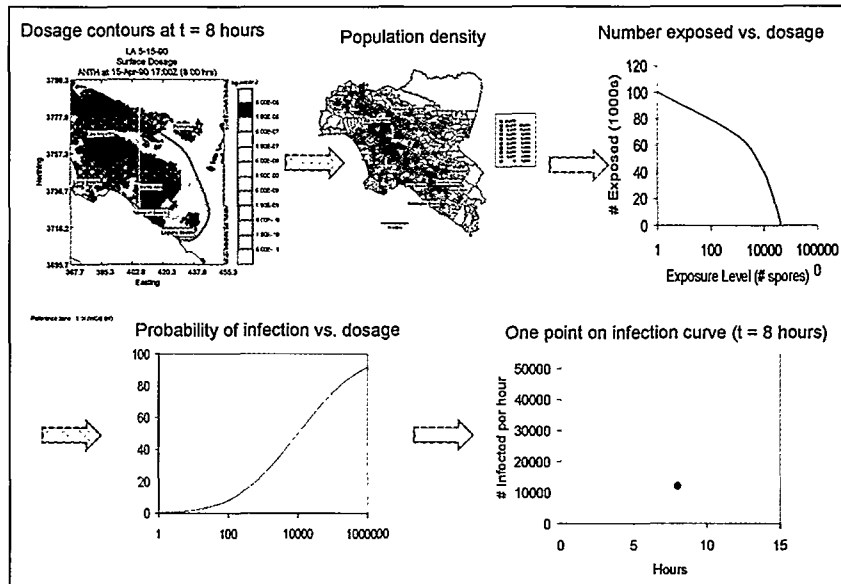
The infection curve represents the infection of the population following the release of a CB agent.

The key result is a map of the cumulative exposure levels of the target population as it interacts with the transported agent. This exposure map is then correlated against the probability of an infection (a statistical relationship between the dose level and chance of infection or poisoning) and integrated over the spatial dimension. The result is a curve that expresses the time history of the infection rate for a given attack scenario. When constructing an infection curve for contagious agents, it is necessary to consider the possibility that the infected victims can in turn infect others within the previously unexposed population. The subsequent generations of infected victims can serve as a multiplying effect and aid the spread of a contagious disease.

The Epidemic Curve

The epidemic curve depicts the expected rate at which the collective infected victims will reveal clinical symptoms following an infection or poisoning event. Since some chemical agents produce rapid symptomatic response, the resulting epidemic curve may closely mirror the progenitor poisoning curve in time (see figure at right). For biological agents, the incubation period between the time of infection and the onset of clinical symptoms produces an epidemic curve that lags significantly the infection curve. Additionally, variations in dose received and differences in individual physiological response to the disease further widen and alter the net shape of the collective epidemic curve. Thus, the master epidemic curve is reflective of underlying medical phenomena for a given agent, and represents the medical expectation of symptoms for statistically significant numbers of victims. For commonly occurring diseases, the epidemic

This timeline curve depicts the release of a notional chemical agent (e.g., sarin). The curve represents results in which no medical or protection system intervention takes place. In contrast to most BW agents, the curve shows the nearly simultaneous occurrence of infection, appearance of symptoms and outcome (death).

curves represent results based on extensive clinical experience and observations. For rarer diseases, the curves represent best fits to documented cases and laboratory data.

The Outcome (Causality/Fatality) Curve

This curve represents the expected outcome (usually fatalities) of populations who have been infected by the agent (see the top figure on page 59). For the agents studied the curve is also based on medical experience and clinical data for a given agent. Thus, the nominal fatality rate curve is reflective of the expected death rate following a standard medical treatment regime based on presentation of symptoms (i.e. corresponding to the epidemic curve). If the epidemic curve is altered due to medical prophylaxis, then the fatality curve will also be altered to reflect the probability of greater survival rates. Other types of outcome curves might be more appropriate for a given scenario or agent. For example, some chemical agents can result in a significant fraction of the people exposed with permanent debilitation. Also, some biological agents are essentially non-lethal, but result in a high rate of sickness and extensive periods of incapacitation. For these kinds of outcomes, the medical and societal impacts will be different and significant to the evaluation of net effects of a CB attack on urban populations.

Interpreting the Master Timeline Curves

The principal value of the master timeline curves lies in their simple but effective means for providing a defenders perspective relative to CB attacks on U.S. civilian population centers (cities and urban areas). The master timeline curves can be utilized as rate curves (i.e., differential curves that describe the time rate of change) or as integral curves (i.e., illustrate the cumulative effect). Rate curves serve to illuminate the importance of potential response and preventive actions on subsequent events and outcomes. Integral curves serve to illustrate the overall magnitude and the potential impacts of a given threat scenario.

The primary significance of the *master infection curve* is that it represents the integrated outcome of a series of inter-related events and complex phenomenology into a simple measure - the rate and cumulative number of people infected in the attack scenario. The curve reveals the time history of the infection process for a given attack and thus provides the baseline for gaining insights into precautionary and responsive measures for reducing the number of people infected. Since the infection rate provides the initial conditions that drive the epidemic and outcome curves, interfering with the infection curve directly translates to reduced casualties and fatalities. Several approaches to interfere with the baseline infection curve are possible. If forewarned of the time, place and nature of an attack, the population could be evacuated or provided with other protective measures. If forewarning is unavailable, then prompt detection of agent in the immediate environment could provide a limited warning time to cue appropriate protection measures. However, any protective actions must be taken within the time-scale of the baseline infection curve itself to be effective in reducing the overall number of infected - thus an understanding of the infection curve provides the overall time budget available for protection measures.

The importance of the *master epidemic curve* is in understanding the effects of responsive medical actions. If environmental detection, identification and warning (EDIW) is available as an attack alarm, then aggressive medical prophylaxis can significantly change the shape and magnitude of the epidemic curve. In the absence of EDIW, medical surveillance will become the first alarm signal that an attack has taken place. Since the epidemic curve represents the rate at which the infected population becomes clinically symptomatic, then positive detection of disease closer to the initial rise of the curve becomes a means for increasing the alarm time. For many diseases, earlier treatment results in higher survival rates. Parametric epidemic curves can be constructed that reflect the effectiveness of carrying out medical intervention and prophylaxis for various delays in warning time following infection. This kind of analysis can provide insights into defensive architectures utilizing combinations of environmental monitoring and medical surveillance strategies.

The *master outcome curve* provides the key metric for gauging the attack and measuring the effectiveness of a defensive architecture in terms of causalities and lives saved. The integral of the fatality curve gives the total number of fatalities expected. Thus, parametric outcome curves when generated from corresponding infection and epidemic curves can be used to assess defensive and responsive strategies with respect to a key metric.

Future Outlook

In Phase II of the Defense of Cities study, the master timeline curve model is being further refined to enable assessment of smallpox releases at both *micro* (specific release in a specified urban area) and *macro* (secondary transmission in other geographic areas) levels. In addition, the model is being applied to characterize other BW agents: plague, tularemia, and Ebola-Zaire. These agents, along with the curves developed for anthrax and sarin in Phase I, will provide a spectrum of agent effects that will be applied to the development of architectures and systems for urban defense. System performance and trade-off analyses will be conducted using the master timeline curve model. This assessment will enable more detailed characterization of specific systems that could comprise urban defense architectures for near-, mid- and longer-term applications. In this analysis, emphasis will be placed on identification of those urban defense system elements that have major leverage in casualty reduction.

Analytical Studies
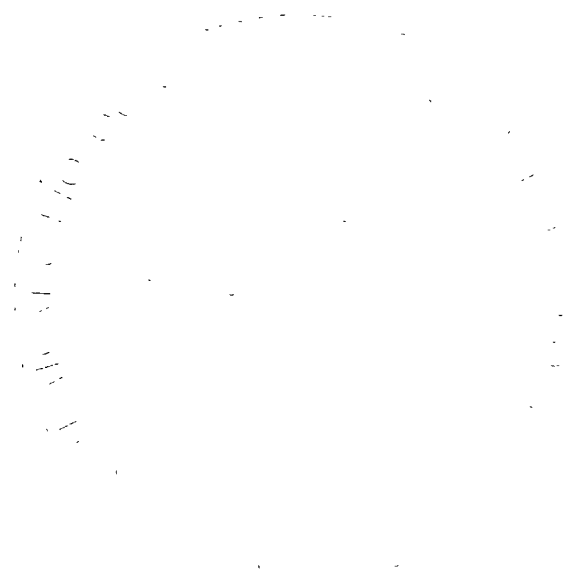
Autonomous Pathogen Detection System

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Objective

The objective of this project is to design, fabricate and field demonstrate a fully Autonomous Pathogen Detector (identifier) System (APDS). This is accomplished by integrating a proven flow cytometer and real-time polymerase chain reaction (PCR) detector with sample collection, sample preparation and fluidics to provide a compact, autonomously operating instrument capable of simultaneously detecting multiple pathogens and/or toxins. The APDS is designed to operate in fixed locations, where it continuously monitors air samples and automatically reports the presence of specific biological agents. The APDS utilizes both multiplex immuno and nucleic acid assays to provide "quasi-orthogonal", multiple agent detection approaches to minimize false positives and increase the reliability of identification. Technical advancements across several fronts must first be made in order to realize the full extent of the APDS. Commercialization will be accomplished through three progressive generations of instruments.

The APDS is targeted for domestic applications in which (1) the public is at high risk of exposure to covert releases of bioagent, such as in major subway systems and other transportation terminals, large office complexes, and convention centers; and (2) as part of a monitoring network of sensors integrated with command and control systems for wide-area monitoring of urban areas and major gatherings (e.g., inaugurations, Olympics, etc.). In this latter application, there is potential that a fully developed APDS could add value to Defense Department monitoring architectures.

The top-level goals of the program are to:

- Develop and field demonstrate an autonomous system utilizing a single-agent flow cytometer assay

- Add multi-agent analysis capability (multiplex detection)

- Add PCR identification capability

- Commercialize the technology

- Partner with first-responder agencies

Recent Progress

Completion of APDS-I system
We have completed the construction of a version 1 prototype APDS (APDS-I) system. This system incorporates an aerosol collector from Research International (RI), a custom-built fluidics system, and a MicroCyte flow cytometer. The fluidic system is
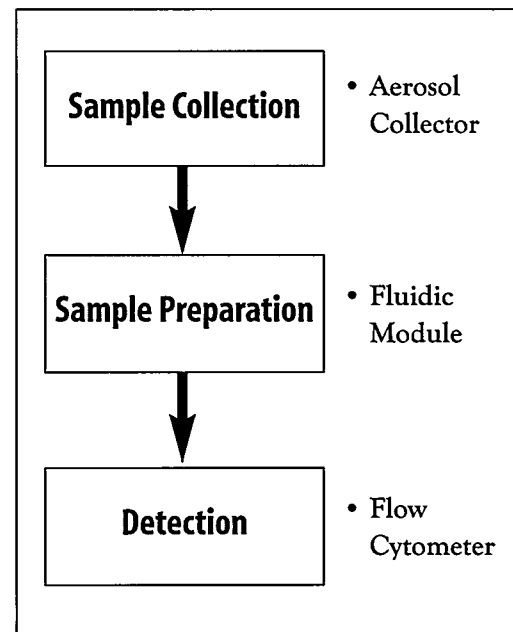
APDS Assay System.

The use of a combination of two detection technologies increases system reliability and reduces false positives.
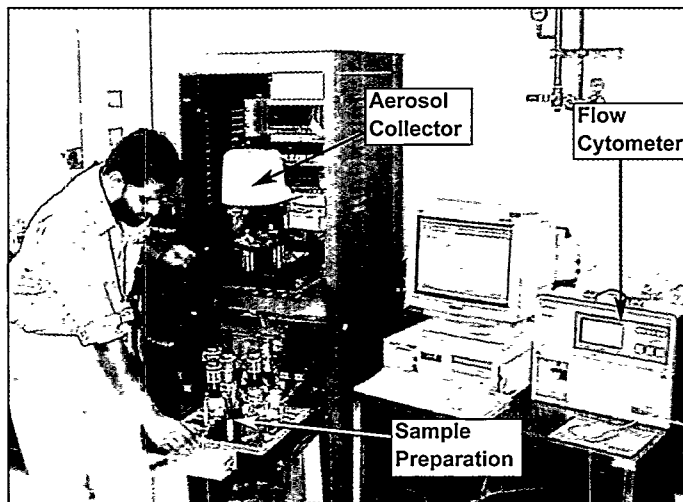
Version 1 APDS Monitoring System.

capable of mixing and dispensing three different reagents for incubation with the sample before delivery to the flow cytometer. Other features still under development include the incorporation of clean cycles and archival storage of positive samples. The control system utilizes LabView for instrument operation, data acquisition and data storage, and to automatically call positive samples. We have preformed several studies to characterize the operation of the APDS-I system and to prepare it for a field trial at the PNNL Wind Tunnel Facility.

Evaluation of the Fluidics Subsystem for the APDS-I

A method using multiple fluorescent beads in the reagent chambers to evaluate the fluidics and flow cytometer was developed. Sequential measurements of the proportion of each bead type test both reagent metering and cytometer performance. This approach has been successfully used to demonstrate autonomous operation of the system for periods up to 24 hours. Fluorescent beads are now added to the assays to provide an internal quality control for system operation.

Immunoassay for B.g.

We have also adapted a direct-labeling immunoassay for *B.g.* to the MicroCyte flow cytometer. A far-red fluorescent labeling method was developed that is compatible with the optical system of this flow cytometer. We have characterized this assay using dilutions of *B.g.* spores in buffer and spores in aerosol collector fluid.

Benchmark Test of the APDS-I System

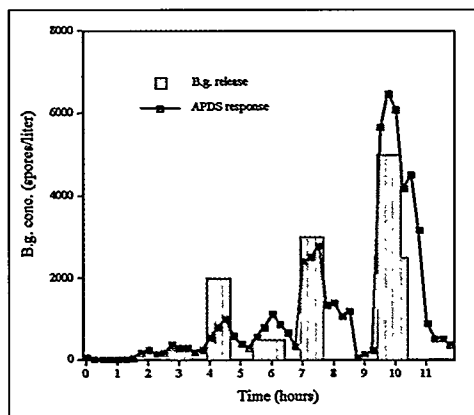The APDS-I system has been taken to the PNNL Wind Tunnel Facility for a field trial to evaluate its performance in identifying *B.g.* aerosols. The system performed successfully in fully autonomous operation for up to 12 hours. Aerosols containing a range of *B.g.* concentrations were used to demonstrate that the APDS-I response was proportional to

The APDS instrument can automatically detect and identify an anthrax surrogate.

spore concentration and that concentrations down to 50 spores per liter of air could be detected.

Evaluation of Advanced Components

We have begun evaluation of advanced components for a second version autonomous detection system (APDS-II) that will allow multiplex operation at increased sensitivity and

12 Hour Continuous APDS Run at PNNL Wind Tunnel.

reliability. The principal components will consist of an LLNL-RI hybrid aerosol collector, a Luminex multiplex flow cytometer, and next-generation fluidic systems.

Future Outlook

We will complete the evaluation of the advanced components for the APDS-II, adapt these components for autonomous operation, and reduce them to practice through the APDS-II instrument. The APDS system will offer true multiplex operation with 1–2 orders of magnitude improvement in detection sensitivity. A second primary objective is to adapt flow-through PCR techniques, currently under development at LLNL, to the ADPS instrument. Thus, the APDS will utilize two complementary technologies for increased detection reliability and identification of pathogens. Finally, we will evaluate emerging technologies, such as the Sandia μChemLab™ and new mass spectrometric techniques, for potential inclusion in future generation systems.

Publications

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Field testing of the APDS at the PNNL Wind Tunnel Facility successfully demonstrated automated operation for 12 hours with results being reported every 20 minutes.

Development of multiplex methods is currently in progress to allow simultaneous detection of many pathogen types.

Detection

µChemLab/CB™: a Hand-Portable System for Detecting a Broad Range of Chemical Agents and Biotoxins

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Detection

Objective

The objective of this project is to develop a fully self-contained, hand portable detection system that can be used in all phases of domestic terrorism scenarios. This system, known as µChemLab/CB™, will simultaneously detect a range of chemical, biotoxin and viral growth media signatures at high sensitivities (1 ppb chemical or 1 agent-containing particle/liter of air, ACPLA, biological) in approximately 3 minutes with extremely low false alarm rates (approximately 0.01%). It also has a 5-second "alert mode" for chemical nerve agents. µChemLab/CB™ will not directly detect bacterial/rickettsial disease agents but will complement the performance of DNA-based detectors under development at the Lawrence Livermore and Los Alamos National Laboratories.

The µChemLab/CB™ detector differs from other detectors under development for CB applications. By taking advantage of advances in microfabrication, separations science, and biotechnology, we are able to create a new type of analytical device that cannot be implemented using macroscopic fabrication methods. At the heart of the device are micromachined chips, each with arrays of parallel and serial microseparation channels (columns). Each column separates compounds on the basis of distinct chemical interactions, allowing multiple different chemical properties of the compounds to be probed. After separation, the compounds are detected using highly sensitive techniques: laser-induced fluorescence for liquid-phase separations, and arrays of gravimetric (surface acoustic wave) sensors for the gas phase. Additional sensitivity and selectivity are added through a variety of preconcentration steps prior to separation. The battery of serial and parallel preconcentration, separation and detection steps creates a detailed signature for each compound based on its unique chemical and physical properties. This selective and information-rich process provides the ability to detect a vast range of chemical and biological compounds in the presence of complex backgrounds. The result is a high-sensitivity chemical sensor with a low false alarm rate. Another important characteristic of this
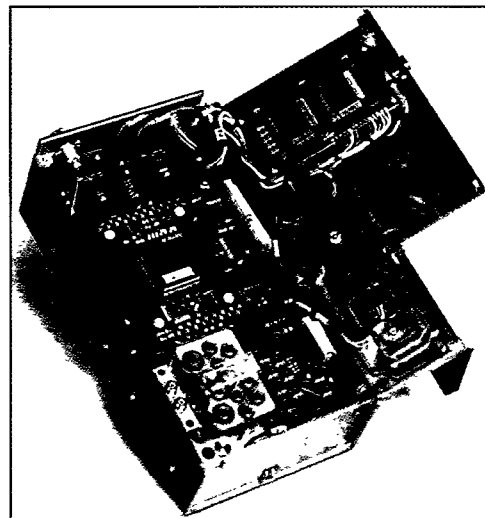

The µChemLab/CB™ is being developed to provide a fully self-contained, hand portable detection system that can be used in all phases of domestic terrorism scenarios.

μChemLab/CB™ is a hand portable detection system that will simultaneously detect a range of chemical, biotoxin and viral growth media signatures at high sensitivities in approximately 3 minutes.

An array of microfabricated sensor components work in parallel and serial configurations to identify CB agents in the presence of complex backgrounds with low false alarm rates.

microscale approach is that the time scales for time of analysis are on the order of a few minutes. These short analysis times result from parallel processing as well as from the intrinsic efficiencies of the physical processes implemented on the microscale.
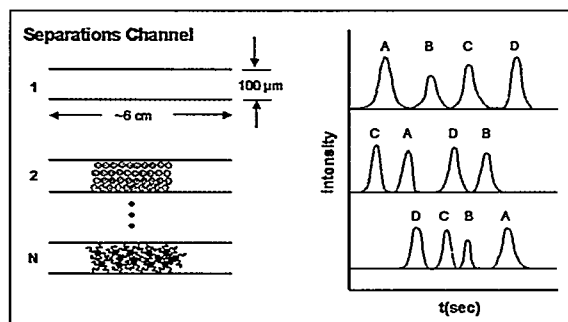
Multiple chromatographic separations are key to broad coverage and low false alarms.

μChemLab/CB™ employs a modular design to facilitate subsequent upgrades. As described below, we are constructing a liquid-phase analysis module for the detection of biotoxins (and growth media signatures) and a gas-phase analysis module for the detection of chemical agents. FY99 has focused on migrating separations and detection techniques, developed using free-standing microcomponents such as capillaries, to systems using integrated microfluidics fabricated using micromachined glass and silicon. FY00 will focus on systems integration of the detector and microseparations systems into the gas- and liquid-phase modules, with the first integrated unit targeted for mid-FY00. Ongoing separations, detection, and microfabrication R&D will support successive generations of units with expanded ranges of agents and improved capabilities.

The expected result of this effort is a relatively universal sensor platform that will provide unprecedented chemical analysis capability that is compatible with the need for extensive low cost distribution and autonomous operation. Top-level goals in towards reaching this end include:

- Demonstration and field test of a first-generation research prototype

- Additional chromatographies and protocols to address a very broad range of agents, including viruses and their associated media

- Rapid transition to engineering and manufacturing prototypes

Recent Progress

Liquid-Phase Analysis of Biotoxins
Key technical advancements were made in FY99 in developing optimized separation and detection methods that have been packaged to work in integrated systems using many microfabricated components. The recent accomplishments are highlighted below.

Separation and Detection

The need to sense very trace quantities of biological toxins presents an enormous technical challenge. Our approach is to divide that problem into two components. In the first step, we use electrokinetic separation methods (separations that use electric fields to invoke molecular motion) to provide selectivity. This step is followed by a detection step that measures the presence of an isolated collection of molecules. In order to meet the needs for BW threats (1 ACPLA) we must be able to measure concentrations in the sub-ppb range that are contained in extremely small volumes (submicroliter). Laser-induced fluorescence (LIF) is one of the few approaches that can achieve such sensitivity. During FY99, we made significant progress on labeling biological toxins with fluorescent tags that allow us to approach these low detection limits. In addition, we developed optimized separation processes that allow the unique identification of the labeled biotoxins. Of particular importance has been the first-ever demonstration of electrokinetically pumped high-pressure liquid chromatography (HPLC) separations of proteins. This powerful separation approach takes advantage of our recent discovery that allows the generation of large pressure gradients in a microscale device. We also demonstrated several preconcentration processes that allow the lowering of detection limits of the selected molecules by approximately two orders of magnitude.

System Integration and Packaging

One of the important aspects of our approach is that the system has the potential to be fully integrated into a compact and low-cost package. We made several major accomplishments in this area. The first is the successful demonstration of macroscale-to-chip connectors that allow replaceable fluid cartridges to be connected to the chip. This system allows rapid replacement of fluids to the chip through an array of O-ring-sealed needles that also act as the electrodes for applying electric fields to the chip. The second accomplishment is the fabrication and testing of a compact high-voltage board that allows variable high-voltage supplies (0–3000 kV) to be applied to a multitude of electrodes. These voltages provide the effective switching of fluidic routing on the microfabricated chip. The third key accomplishment is the refinement of the microfabricated structures themselves. Detailed optical measurements and computer modeling allowed new and optimized fluid networks to be constructed that optimize the speed and selectivity of the microchip based separations.

Gas Phase Analysis of Chemical Agents

The gas-phase module contains multiple chemical analysis channels that incorporate three main microfabricated components:

- Membrane-based preconcentrators (PC) that are coated with high-surface-area adsorbents,
- Gas chromatographic (GC) columns

Detection of chemical agents makes use of selective chemical thin films that enhance sensitivity and discriminates against the vast number of harmless background gases.

- Chemically selective detectors based on arrays of surface acoustic wave (SAW) sensors.

Work has focused on tailored porosity thin-film adsorbent layers for the preconcentrator, development and optimization of stationary phase coatings for the GC column, and building and operating a vapor delivery system for component and system testing. Current work has progressed to include fabrication and testing of a breadboard system consisting of the three microfabricated components. This testing has included chemical challenges from nerve and blister agent simulants as well as common interferant chemicals. Key accomplishments this year include:

Coating Technology

We developed hydrophilic and hydrophobic mesoporous sol-gel coatings for deposition onto the membrane preconcentrator. Spray-deposited films have allowed us to demonstrate concentration factors of over 100-fold for a 30-second collection time using dimethyl methyl phosphonate (DMMP) as a nerve agent simulant.

High sensitivity is achieved through "weighting" the molecules using an array of microfabricated devices referred to as surface acoustic wave detectors.

Gas Chromatography Columns

Micro-GC columns have been coated with a variety of coatings from a nonpolar polydimethyl siloxane (OV1) to a very polar polyethylene glycol (Carbowax). These coatings have been deposited on a variety of planar micro-GC columns using a modified sol-gel technique that chemically bonds the phase to the column surface. Multiple columns exhibiting unique separations have been demonstrated for use in a multichannel analysis system.
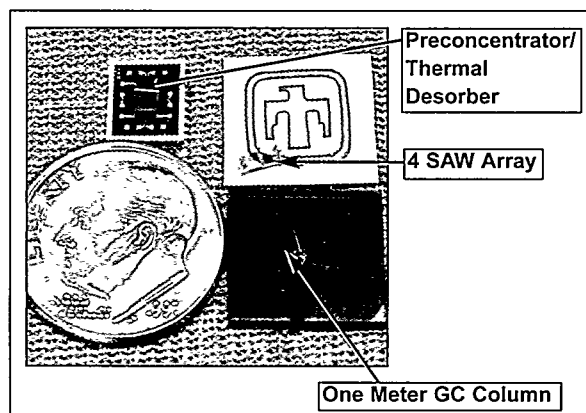
Clockwise from upper left: Preconcentrator/Thermal Desorber, four-element SAW Array, and one-meter GC Column.

System Integration and Packaging

Breadboard tests with all three components linked together have shown excellent selectivity for nerve agent and mustard simulants in the presence of large amounts of interfering compounds. The micro-GC column has separated sarin and soman simulants from each other as well as from a mustard simulant and from common interferants.

Future Outlook

μChemLab/CB™ is being implemented initially for a high-profile subset of biotoxin, biological warfare (BW) and chemical warfare (CW) agents. As described above, FY99 activities focused on transitioning the critical technologies from the laboratory bench to chip, demonstrating the necessary sensitivities, and beginning the integration of the gas-phase and liquid-phase analyses modules. In FY00, we will complete the development of the analyses modules and will integrate them into a research prototype unit, complete with power systems, control systems, data analysis and I/O. The initial unit, targeted for third quarter FY00, will combine both gas-phase and liquid-phase modules. Concurrently, we will enhance the capability through development of the additional liquid-phase separation methods, which will add reverse phase, size exclusion, and ion-exchange chromatography to the arsenal for low false alarm detection of biotoxins. Out-year efforts will expand the range of BW agents to include a broad range of large protein toxins, the Priority 1 list from the Chemical Weapons Convention, and selected vapor-phase species that are signatures of BW agent production or dispersal. A second-generation research prototype covering the expanded range of biotoxin and chemical agents will be available in FY02 and a viral detection module added in FY03.

> The μChemLab/CB™ system is designed to have unprecedented chemical/biochemical selectivity and sensitivity in a format that will eventually allow distribution at a low cost per device.

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Detection

Advanced Ion-Trap Mass Spectrometry for Detection and Identification of Chemical/Biological Threats

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Objective

The objective of this project is to develop novel methodologies and hardware based on a suitcase size, fieldable quadrupole ion-trap mass spectrometer platform that makes possible the detection and identification of the full range of chemical and biological warfare threats. The system provides direct atmospheric sampling mass spectrometry for rapid, real-time detection of airborne bacteria or volatile organics, either operator-directed or as a stand-alone monitor, combined with a capability to analyze collected samples with higher specificity by a novel mass spectrometric approach. Potential end-users include members of the security, law enforcement, and emergency response communities. The instrument will provide rapid on-site identification in the field with short turnaround times. Such a capability will be valuable in minimizing false positives, thereby saving time and money, and minimizing false negatives, thereby saving lives. The size of the units would permit them to be transported to locations where releases are suspected or anticipated. It is expected that with the aid of one of these instruments, an emergency response team could investigate and resolve most hoaxes and accidental releases with little additional assistance and could participate effectively in handling a large release. Depending upon the scenario, the field ion-trap system can play roles in detect-to-warn, detect-to-treat, and detect-to-restore applications.

Recent Progress

Progress in FY99 included the interrogation of airborne particles (bacteria, viruses, and natural background), the use of electrospray and ion/ion chemistry for protein biomarker detection, and the development of an improved ion-trap geometry based on a toroidal design.

Real-Time Airborne Particle Mass Spectrometry
The airborne particle mass spectrometer is an instrument that performs gas-phase analysis of individual airborne particles on a real-time basis. The spectrometer uses laser ablation together with ion trap-mass spectrometry to analyze individual airborne particles as they enter the instrument. It operates on demand, i.e., each incoming particle triggers the laser ablation–mass analysis process. Thus the instrument could be used as a stand-alone monitor to detect changes in the particulate background or as an

> With this instrument, an emergency response team could investigate and resolve most hoaxes and accidental releases with little additional assistance.

Mass spectrometry can detect biological as well as chemical weapons threats.

The instrument can be used as a stand-alone detector or operator-driven device to detect the presence of biological threats.

operator-directed survey instrument to detect the presence or absence of airborne bacteria. A laboratory version of the airborne particle mass spectrometer has been used to acquire single particle mass spectra of a wide range of samples (six species of bacteria, one bacterial spore, six species of pollen, six NIST standard reference particulate samples, and three Dugway simulants) to aid in the development of identification methodology.

We have teamed with Sandia National Laboratories to explore the use of intelligent computer algorithms for the detection and/or identification of individual bacteria from the mass spectral data generated by this instrument. The approach we have taken for particle classification or identification is the feed-forward neural network. Neural networks can be assembled and trained to give a binary output, separating the input data into two classes. The success of the airborne particle mass spectrometer as a bacterial counter depends on its ability to make a classification decision for each particle.

In our neural net experiments with averaged spectra, the six bacterial species could be successfully discriminated from all of the other types of particles. In our most recent tests, a neural network trained with averaged spectra was then challenged with single particle mass spectra from the library. This means that, in principle, our instrument can now make an unassisted real-time classification decision (still not always correct) on each incoming aerosol particle that is analyzed as to whether or not it is a bacterium. Although this preliminary experiment yielded some errors on individual particles, false alarms can be reduced substantially by combining the results for several particles.

Electrospray Ion/Ion Chemistry

We are also exploring a novel strategy for the detection of proteins from pathogenic bacteria, viruses, and bio-toxins using electrospray and ion/ion reactions. In the interest of speed and simplicity, it is desirable to minimize sample pretreatment and separations. The ability to extract and analyze unique protein molecular signatures from BW threats in a wide range of matrices (e.g., air, water, surface wipes) will be coupled with identification using either existing genomic/proteomic databases or custom-built libraries.

A key component of this work is the generation of partial-protein sequence information from intact proteins. This information, called a "sequence tag," can then be used to effectively identify a targeted protein, which in turn can identify a given species of microorganism (e.g., bacteria or virus). This technique would also be effective in identifying unknown proteins/microorganisms (e.g., any genetically designed bioterrorism bacteria or virus).

We have characterized a wide variety of proteins from different organisms including disulfide-linked and reduced proteins, viral coat proteins, protein toxins, and crude extracts of bacterial proteins. We have successfully applied this technique to the analysis of the Dugway Proving Ground simulant bacteriophage MS2. In this case, the sequence tag information was generated in approximately one minute, with a computer search time through a typical protein database of five seconds.
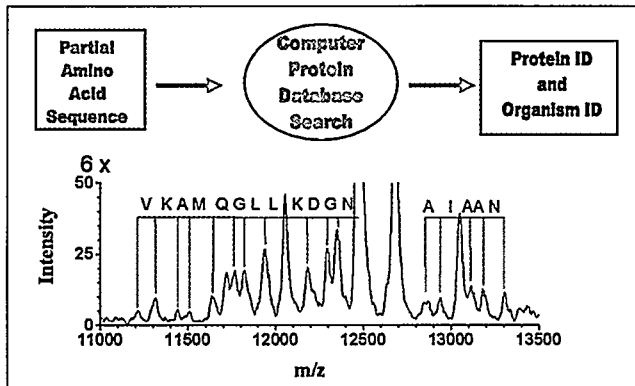
Peptide Sequence tag (one letter amino acid codes) for the Dugway Proving Ground simulant bacteriophage MS2. The partial amino acid sequence of a protein can be used to identify not only the protein of interest, but the microorganism from which it was derived.

Proteins can be used to identify known and unknown biological threats.

Toroidal Ion-Trap Analyzer

Small instrument size, simple ion optics, ultrahigh sensitivity, and the selectivity of MS/MS are just a few of the reasons that ion-trap mass spectrometry has gained popularity for use as fieldable mass spectrometers. Efforts to further reduce size, weight and power consumption of the ion-trap are favorably governed by the inverse relationship between the analyzer radius and the amplitude of the RF trapping field. Unfortunately, smaller ion-trap analyzers are more prone to performance degradation arising from space charge (ion-ion) repulsion. A new ion-trap analyzer geometry has been developed where the ion-trapping field is in the shape of a torus. This toroidal ion-trap geometry allows the design of a significantly smaller ion-trap system while still maintaining the storage capacity of a standard ion-trap. An early version of the toroid analyzer was constructed from a standard, symmetrical ion-trap cross-section. The mass analysis performance of this analyzer was characterized by broad, poorly resolved mass peaks. In this early version, no attempt was made to correct for field imperfections that would be introduced from the rotation of the ion-trapping field. More recently, trapping field analysis programs such as POISSON/SUPERFISH (Los Alamos National Laboratory) and ion-trajectory simulation programs such as ITSIM (Purdue University) and SIMION (Idaho National Engineering and Environmental Laboratory) have been used

New developments in ion-trap analyzers will increase performance for the next generation biodetector.
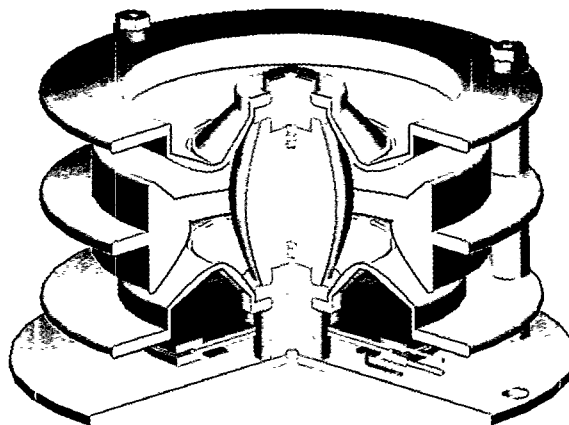
to optimize the shape and then characterize the resulting ion-trapping field. The resulting asymmetric analyzer design should offer dramatically improved mass analysis performance and allow the toroid analyzer design to achieve its predicted performance level.

Future Outlook

Results to date offer promise for the development of a simple-to-use system for the detection and reliable identification of both chemical and biological threats. The program builds on an existing ORNL program with the U.S. Army to develop a battlefield CW/BW detector, the Block II Chemical and Biological Mass Spectrometer (CBMS-II). The remaining instrument challenges include the development of a field-hardened electrospray ion source, the integration of the electrospray ion source and sampling handling system into the existing CBMS Block II control system, and the development of the operator control interface. Transitioning the electrospray protein identification approach from the laboratory into a pushbutton instrument usable by emergency responders or other civilians is the key remaining challenge for this project. This includes the optimization of protein sequence information from the electrospray mass spectrum of proteins as well as the development of database screening algorithms.

Experiments/Field Testing

Extensive laboratory and field testing on both simulants and threat targets are integrated throughout the entire program. The prototype electrospray-based system will be compared with the current pyrolysis-based CBMS II for a set of biosurrogates sometime late in FY00.

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Detection
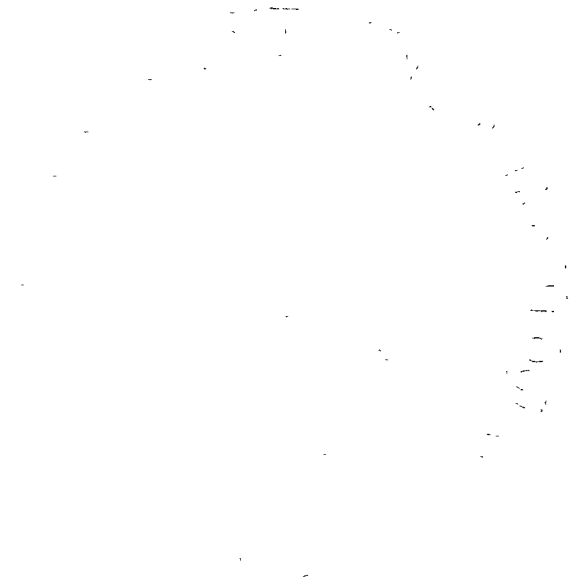
Bacterial Fingerprinting by DNA Fragment-Sizing Flow Cytometry

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Objective

The objective of this project is to develop new instrumentation and supporting methods for fingerprinting of bacteria by DNA Fragment-Sizing Flow Cytometry. This approach combines the reproducibility and reliability of Restriction Fragment Length Polymorphism (RFLP) analysis with the speed and sensitivity of flow cytometry analysis.

In order to identify bacteria by DNA fragment sizing analysis, pathogen genomes are digested with rare-cutting enzymes and the resulting set of fragments is stained with a DNA intercalating dye that binds stoichiometrically to each fragment. The fluorescence intensity from each DNA fragment is then measured and is directly proportional to the number of basepairs comprising its length. The "fingerprint" determined in this way varies by species, by strain and by restriction enzyme. Sequence information about the bacterial sample is not required, allowing the identification of unknown, newly emerged or unanticipated strains. This technology is applicable to the discrimination among species and strains of human pathogens and will be an important tool in determining the causative agent in an outbreak involving a bacterial pathogen.

Recent Progress

During the past year, bacterial strain discrimination was demonstrated using our methods on a wide variety of bacteria. The sample protocols were greatly simplified, shortened and verified for accuracy. A compact version of the DNA fragment sizing flow cytometer instrument was made more robust and user-friendly. Protocols for staining DNA fragments were developed to take advantage of available wavelengths on small solid state lasers. A prototype version of software for matching results of unknown test samples to a database of DNA "fingerprints" was developed.

The fluorescence signal for three biological weapon simulants.

Future Outlook

During the next year we will be seeking a commercial manufacturer for the DNA Fragment Sizing Flow Cytometer instrument. Further studies will be done to validate the sample preparation protocols for a selected set of Gram-negative, Gram-positive, and spore-forming bacteria. Finally, the database of known DNA fingerprints will be expanded and tested.

Biochromic Conjugated Polymers

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Objective

The objective of this project is to develop biosensors using biochromic conjugated polymers (BCP) for instant detection of biological pathogens and biological warfare agents for use in the event of biological attack or suspicious outbreaks. The sensors are designed to be capable of multiple signal output with high sensitivity and low false-positive response. This project is based on the one-step colorimetric detection technique previously developed at LBNL in which BCP sensors change color from blue to red upon binding of a biotarget such as virus or toxin. The BCP sensors are integrated biosensing units in that molecular recognition, amplification and signal transduction are encompassed in a single self-assembled microstructure, and the method does not require tagged antibodies, separation steps or secondary visualization reagents. Although rapid and easy to use, the colorimetric method suffers from limited sensitivity and stability. The goals of our research are to enhance the sensitivity, broaden the working range and lower the false positive response rating of BCP-based sensors through the use of multiple "quasi-orthogonal" detection techniques (QODT), namely colorimetric, fluorescence and electrochemical methods all incorporated on a single sensor chip.

Recent Progress

Progress to date has included successful synthesis of new BCP materials for optical reporting, development of novel lipid microstructures as sensing matrix and development of electrochemical sensors for bacterial toxins as part of QODT.

Novel BCP Materials for Pathogen Sensors

One of the challenges in sensor development is the lack of appropriate BCP materials that can provide desired optical properties for signal transduction. Material synthesis towards new chemistries with improved optical properties is among the priorities of this project. Our research emphasizes developing novel chemical architectures for amplifying biomolecular binding through introducing new chemistries in the headgroup region (where binding occurs) as well as in the hydrocarbon region (where signaling occurs). Headgroup manipulation allows increased interactions between the molecular component on the lipids and the biological pathogens. The optical properties of the supramolecular assemblies are enhanced as well. A novel system based on hydrazide derivatives of single-chain diacetylene lipids has been synthesized and studied. These materials show an unusual aggregation and polymerization behavior in organic solution, in contrast to the parent carboxylic acids. In addition, these hydrazide lipids undergo an

BCP sensors change color from blue to red upon binding of a biotarget such as virus or toxin.

New materials exhibit promising optical properties for advanced sensors.

unprecedented reversible color change (blue/red) in polymerized vesicles. The findings provide detailed insight into the mechanism of colorimetric transitions in polydiacetylenes (PDA) upon changes in the layer packing. The development of these insights is necessary for the ultimate construction of reversible sensors with enhanced sensitivity. By tailoring the headgroup interactions via hydrogen bonding, the colorimetric response can be controlled in a very effective way. In addition, the ability of the hydrazide lipids to polymerize in solution provides new routes for the synthesis and processing of PDA beyond that of the solid state.

Lipid microstructures are another system that shows promising properties as a sensing matrix. The colorimetric PDA sensors were usually constructed by self-assembly of diacetylene lipids to form bilayer vesicles or films. We synthesized a series of amino acid diacetylene lipids that form novel PDA microstructures including tubule, helix, ribbon, sheet, braided fiber and planar platelet structures. These microstructures exhibit interesting optical properties, and their stability is far more superior than bilayer vesicles. Comparison between thermochromic and pH-induced (i.e., surface charge) transition in microstructures and correspondent bilayer vesicles has been made. Application of microstructures in sensor development is under way.

Ultra-Sensitive Electrochemical Sensors

The center of the project is developing sensors for pathogens. In addition to continuing our pursuit of colorimetric sensors using known and newly acquired BCP materials, we are also exploring pathogen sensors with fluorescence and electrochemical detection aiming at higher sensitivity. A novel biosensor for amperometric detection of *Escherichia coli* enterotoxin has been successfully developed that couples cell surface receptor with redox supramolecular assembly on a sol-gel thin film electrode. The sensor utilizes an open platform to host biosensory elements, allowing fast access of the target molecules to the redox vesicles for detection. The method can detect toxin at far better than colorimetric detection. This work opens doors to the design of effective electrochemical sensors for biological molecules such as proteins and toxins whose detection has been relying heavily on optical signaling as a result of lack of electroactivity. With minor modification, the system can be quickly adopted to developing virus sensors and various immuno-based sensors. The work is also a step forward in the development of ultrasensitive QODT for pathogens and CB agents.

Future Outlook

BCP and QODT provide simple solutions for speedy detection of biological pathogens in the event of biological attacks or outbreak. However, the current form of the sensor is not satisfactory in terms of

detection limit, usable lifetime and portability. Future efforts will be directed towards better understanding of color propagation in the BCP materials upon their binding of a target agent. Superresolution microscopic methods will be used to explore the mechanism of this color transition. In addition, we will seek to add fluorescence detection pathways through synthesis and molecular design of additional materials.

The ultimate challenge is to put BCP films along with multiple detection techniques onto a single sensor chip. We will develop a strategy that allows us to deal with the problem in a progressive fashion. Initial attempt for the QODT method will be tested on commercially available indium tin oxide substrates, using both fluorescence and electrochemical detection modes. At the final stage of the project, a compact sensor with colorimetric, fluorescence and electrochemical detection will be available for testing.

An ultrasensitive sensor has been developed for detecting bacterial toxins at low concentrations.

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Detection

Microsphere-Based DNA Analysis for Pathogen Point Detection

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Objective

The objective of this project is to develop a general analysis platform, based on microspheres and flow cytometry, to enable the simultaneous detection of multiple DNA signatures from multiple organisms. Current assay technology requires organisms to be identified on the basis of a single DNA signature, raising the possibility of high false positive signals. The highly multiplexed assays developed here will employ a high degree of redundancy to provide specificity combined with low false positive and false negative readings. Because the assays are designed to be compatible with a wide range of commercially available laboratory and field instrumentation, we will provide sophisticated analysis capabilities in a kit format suitable for personnel ranging from first responders in the field to health professionals in the clinic.

Recent Progress

Progress to date has included the design of polymerase chain reaction (PCR) primers for several target sequences within the *Bacillus anthracis* genome and associated plasmids as well as the optimization of multiplexed PCR conditions for the simultaneous amplification of all targets. In addition, we have designed and optimized conditions for multiplexed single-base extension (SBE) of hybridization probes to all of the amplified targets. The result is a very robust assay that can detect fewer than 100 copies of *B. anthracis* DNA in a sample by simultaneously analyzing the presence of six different DNA signatures. The assay readily distinguishes *B. anthracis* DNA from that of closely related *Bacillus* species.

Future Outlook

A major activity for the coming year will be to convert the two-step PCR/SBE protocol to a single step PCR/5'-nuclease protocol, which should cut sample preparation time in half. In addition, we will continue to improve assay specificity as well as expand our testing of environmental samples. The assay approach being developed here is very general, and signatures and multiplexed assays are under development for a number of organisms.

We are using color-coded microspheres to simultaneously detect several DNA signatures for each organism.

Developing Automated Amplified Fragment Length Polymorphism Analysis to Identify Microbial Species and Strains

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Objective

The primary objective of this project is to develop an automated, rapid, user-friendly approach to identify pathogenic microbes to the species and strain level. Amplified Fragment Length Polymorphism (AFLP) analysis provides an excellent method of rapidly interrogating a microbial genome to provide phylogenetic information. The method permits analysis of many more genetic loci than is possible using other methods, providing significantly more resolution than methods that rely on comparative DNA sequencing of individual loci. Such an analysis allows discrimination below the species level. When genomic sequences are not available, it can also be used to identify loci that are the basis of DNA fingerprinting systems using multiple locus VNTR analysis (MLVA). To accomplish our goals, we must:

- Develop the necessary software to accurately read the AFLP profiles

- Tie this analysis to computational methods that interpret these phylogenetic data

- Populate an archive with AFLP profilesfor all of the threat agents and phylogenetically related species

Recent Progress

Populating the Archive

AFLP analysis is limited by the number of available profiles from different microbial species and strains that are available for comparison to an uncharacterized sample and by the availability of computational methods that allow rapid comparison of a large population of different profiles and phylogenetic analysis based on these comparisons. We are developing AFLP profiles for specific threat agents and their closest phylogenetic relatives with an emphasis on those phylogenetically related microbes that most likely would interfere with a rapid identification of a biothreat agent.

We have almost completed AFLP analysis of a sufficient number of *Bacillus* species and strains to allow rapid placement of previously uncharacterized *Bacillus* isolates within a detailed phylogenetic tree of this genus. There is a clear distinction between any *B. anthracis* isolate and any other *Bacillus* characterized so far. However, our most recent detailed analyses demonstrate that *B. anthracis* is not genetically peculiar among the *B. cereus* subgroup but is closely related to some members of this group (see figure on following page). We have also completed AFLP analysis of all available *Yersinia pestis* isolates and conducted a comparison of these profiles to those of other closely related

> This technique provides a clear distinction between any *B. anthracis* isolate and any other *Bacillus* characterized so far.
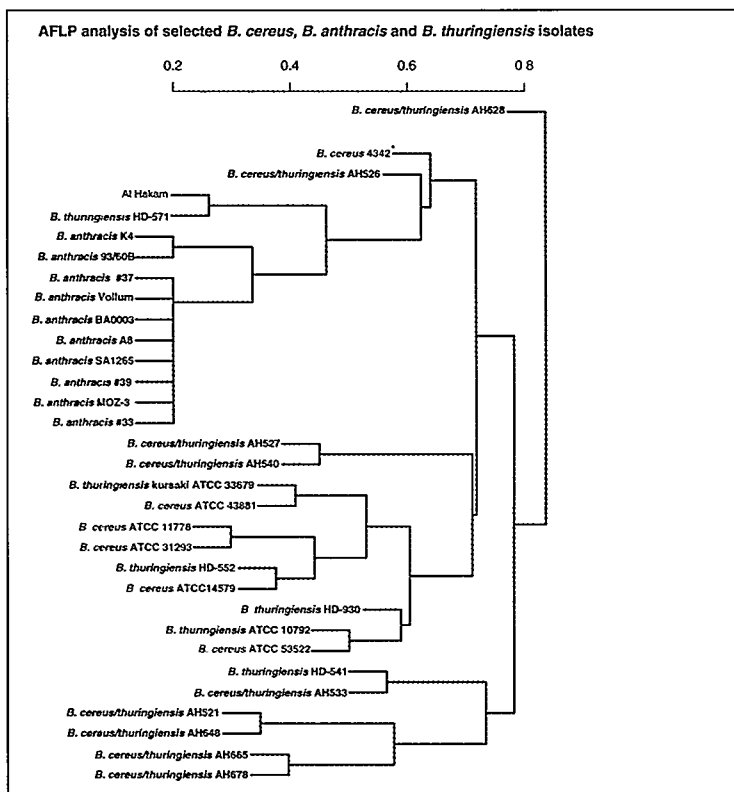
AFLP analysis of *B. anthracis*, *B. cereus* and *B. thuringiensis* strains.
AFLP analysis of different environmental Bacillus isolates demonstrates
that several *B. cereus* and *B. thuringiensis* isolates are closely related
to *B. anthracis*. At least one of these, *B. cereus 4342*, is a known human
pathogen. Al Hakam refers to an UNSCOM sample collected in Iraq.

species. A similar analysis has been initiated on *Clostridium botulinum* and
is only limited by the availability of representative strains of this species.
There is a relationship between the phenotypic characters used to distin-
guish strains of this species and the AFLP profiles obtained for these.
However, this relationship does not extend to the toxins present within
an isolate. The presence of a specific toxin cannot necessarily be predict-
ed by the phylogenetic information, suggesting that the ability to produce
a specific toxin can be transferred laterally to other strains without trans-
fer of a large portion of other genetic information.

Computational Analysis

As the number of AFLP profiles to be compared increases, it is impossi-
ble to do this rapidly using manual methods. We have developed com-
putation methods to compare large numbers of profiles and determine
their phylogenetic relationships. Computational approaches are focus-
ing on methods of rapidly comparing profiles to one another and to
profiles generated for previously characterized microbial isolates. This is
a difficult problem because of minor differences among different gel
profiles and the inability of current software packages to accurately
identify DNA fragment sizes. However, significant progress has been

made. It is now possible to rapidly compare profiles generated from a single new isolate to the large number of profiles in our archive and identify a small number of archived profiles that are similar or identical to the new profile. If an AFLP profile from a test sample that is represented in the database is compared to the archived profiles, 73% of the time the software identifies a single AFLP profile that matches, 16% of the time it identifies two possible matches and 4% of the time it eliminates all but three profiles.

Technology Application

We have applied this technology to analysis of multiple microbial samples provided by other sponsors and collaborators. A thorough analysis of a large *Bacillus* collection using this archive and software provides new information about the relationship of *B. anthracis* to its closest relatives and about the phylogenetic relationships among all members of this *Bacillus* subgroup. The figure on the previous page demonstrates the utility of this approach to understand the complex relationships among different microbial species.

Future Outlook

More work is required to continue populating the archive, to make the software more user-friendly, to improve the connection between the archive and the newly generated profiles, to improve resolution of the analysis algorithm, and to allow automated phylogenetic analysis based on the profile comparisons. However, we have now demonstrated that it is possible to automate the entire process. As computational methods have improved, it is evident that a small number of the profiles in our current archive are not satisfactory and must be reentered. We have developed a method that detects unsatisfactory AFLP signatures and will replace these profiles with newly generated data. We will also continue populating the archive with AFLP profiles for representative samples of all the other microbial threat agents. We have recently demonstrated that a modification of our current methods allows detection and characterization of viruses. When possible, we will generate and archive profiles for the different viral agents. When the viral genome sequence is known, we can predict the AFLP pattern. Deviations from this pattern suggest the isolation of new strains or intentional manipulation of the pathogen.

More work is needed on computational aspects of the project. Database search routines will be further polished and experimental AFLP data can be compared to "theoretical" AFLP results based on genomic sequences of some microbes. We can also improve the analysis routines including clustering, distance measures and tree techniques. We will also increase the search speed. We must also collect the available information on all the samples (i.e., source, geographic origin, pathogenic or

A thorough analysis of a large *Bacillus* collection using this archive and software provides new information about the relationship of *B. anthracis* to its closest relatives and about the phylogenetic relationships among all members of this *Bacillus* subgroup.

other phenotypic characteristics) in the archive and develop the computational methods to rapidly tie this to the phylogenetic analysis. As more samples become available from other sources, these will be analyzed and compared to the current archive to better understand these samples and to better understand the phylogenetic relationships among pathogenic and nonpathogenic microbes. All newly analyzed samples will themselves become part of the archive so that collection of specific strains from different sources can rapidly be detected.

AFLP analysis provides information about which DNA fragments are most variable among different closely related species and among different strains of the same species. This information can be used to identify DNA fragments that most probably will be species-specific. The DNA sequences of such fragments will then provide information for development of pathogen-specific PCR primers. Fragments that vary among different strains of the same species provide a source of information for development of PCR primers that distinguish among different strains of the same species. AFLP analysis requires analysis of purified DNA from a single source. PCR analysis with strain-specific primers provides information of complex sample content without the necessity of purifying a single microbe from the complex mixture. Such primers have been used to analyze complex forensic and environmental samples.

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Development of Genetic Signatures for Identification and Typing of Biological Threat Agents

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Objectives

Rapid and accurate detection and typing of biological threat agents are essential to respond appropriately to the release of potentially harmful microorganisms. These approaches are important not only for more immediate measures, but also for later forensic analysis and attribution. The purpose of this program is to find and exploit differences in the genetic material among BW agents and their relatives to allow rapid and sensitive detection and typing. These differences can be used to accurately discriminate among similar microorganisms, a crucial aspect of an accurate test. Two major types of discrimination are desirable. The first is the ability to distinguish threat agents from very similar harmless microbes that are often normally found in the environment. Erroneous detection of benign close relatives without the ability to clearly distinguish them from a biological weapon would lead to false results thus rendering the test useless. At the same time, the test must allow detection of the threat agent even though there can be considerable diversity within species of microorganisms. In other words, a test that misses detection of a given agent on occasion is of limited value. The second type of discrimination is the ability to group microorganisms within a species once an organism has been identified. This ability is essential for forensic analysis and attribution. Even though they may cause the same disease, strains within a species can vary based on such factors as geographical origin and the time taken from the environment (e.g., an infected host) into the laboratory. A test that can exploit these differences can produce patterns or signatures that can be used to match or distinguish different isolates of a particular agent. This information could be used to narrow the likely sources of a particular strain. Also, a released agent could be matched with a high level of confidence to organisms acquired through subsequent legal investigation.

Using the genetic material (nucleic acids) of microorganisms as a basis for signatures has several advantages over other typing methods. Most often, the genetic material is in the form of DNA, but in the case of some viruses is in the form of the less stable DNA relative, RNA. This material is often very stable and is present even when the organisms are no longer alive. Also, nucleic acid detection methods such as the polymerase chain reaction (PCR) are extremely sensitive, relatively easy to optimize compared to other methods, highly reproducible, and the test-specific materials necessary are easy to generate and store. The principal goals of the project are:

- Identification of genetic differences among microbial threat agents and non-pathogenic relatives

- Identification of genetic differences among different groups within a particular agent

- Database analysis to define signatures based on these differences

- Development of laboratory protocols to facilitate the use of such signatures

- Development of high-throughput methods to increase efficiency, better methods of database analysis, and a central database for the standardized use of multiple laboratories and organizations.

Recent Progress

Using a technique known as subtractive hybridization, DNA from a given microorganism can be compared to DNA from a close relative and segments that are unique to the pathogenic agent in question can be isolated by the removal of segments that are common. In the case of some viruses that use RNA as the genetic material, the RNA can be converted to DNA for the purposes of these experiments. The specific structure, or sequence, of the unique DNA segments can be determined and based on this information another technique, the polymerase chain reaction (PCR), can be used to detect the presence of minute amounts of a given segment and therefore the organism which carries it. In any such comparison, these segments may or may not correlate well with a given agent but rather may be found in another close relative or may be absent in other sources of the pathogen. Therefore, the presence or absence of the various DNA segments must be tested in a wide variety of strains and closely related species to find those segments that are truly agent-specific. Similarly, strains within a species can be group into different subspecies or biovars based on a variety of biological tests. When strains are compared by subtractive hybridization, PCR is used to test a wide variety of strains and closely related species to find biovar specific markers. The results of all these tests is then combined and analyzed to find candidate signatures. Subtractive hybridization has many advantages over other approaches to signature development. Small amounts of DNA are adequate and are often available from collaborators, thus obviating the need for specific expertise of a given organism. The genetic makeup of the organisms does not need to be characterized. Finally, the likelihood of developing specific signatures is maximized as all the genetic material of the organism participates in the comparison, rather than other methods that focus on specific regions.

This process has been applied to compare two closely related pathogens, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, both close relatives of the plague agent, *Yersinia pestis*. Although these bacteria are highly similar, they cause different diseases, are found in different hosts, and are transmitted differently. In spite of the similarities, multiple subtractive hybridization experiments have yielded a great

Using a technique known as subtractive hybridization, DNA from a given microorganism can be compared to DNA from a close relative and segments that are unique to the pathogenic agent in question can be isolated

many markers specific to either Y. *enterocolitica* or Y. *pseudotuberculosis*. In addition, strain specific markers were also identified within each of these species. These studies more recently have been expanded to include *Yersinia pestis*. Various strains of Y. *pestis* originating from different locations around the world were compared and strain-specific signatures were developed. Many of the markers were also tested against Y. *enterocolitica* and Y. *pseudotuberculosis*, yielding pestis-specific markers. As a powerful and dramatic demonstration of the validity of the overall method, these experiments led to a single test that could both detect and distinguish these three species without detecting closely related species such as Y. *pestoides*.

There are a great many subspecies within *Salmonella enterica*, including the pathogenic strains that are well-known causes of food-borne illnesses. One such subspecies, enteritidis, is now the most common cause of salmonellosis in the U.S., and was used to deliberately infect 751 people in Oregon in the 1980's in an attempt to influence voter turnout in a local election. Individuals are normally infected through undercooked chicken eggs. The above procedures have been used to find enteritidis-specific diagnostic markers that identify all but a few rare strains of enteritidis and do not detect an extensive array of the many closely related biovars that are common in the environment but do not infect eggs. In addition, these markers are not detected in the great many close relatives of the genus Salmonella that are found in the same environments. In addition to the potential benefits of providing an excellent diagnostic test for monitoring egg production, these results again demonstrate the power of these techniques because in spite of the large number of closely related species and subspecies, a well-tailored signature was developed.

Concomitant with the achievements described above, the efficiency of these approaches has been greatly improved through automation and increased parallel processing of samples. This will spur an increased rate of growth of the signature database.

Future Outlook

Further progress in signature development can be divided into three main areas:

- Additional increases in efficiency of the techniques.

- Development of a central database as well as improved methods for computer data analysis.

- Continued development of signatures for particular threat organisms.

Homology between *Bacillus anthracis* Plasmid-Encoded Genes and Other Bacterial Species

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Objectives

The goal of this project is to identify regions of gene homology between genes encoded on the pX01 and pX02 plasmids of B. anthracis, other *Bacillus spp.* common in the environment, and other pathogenic bacteria, to improve specificity of DNA-based detection methods for B. anthracis in environmental samples, and to determine potential gene function of new genes. We are using a combined DNA hybridization and PCR approach to identify regions of homology between the known B. anthracis virulence genes and closely related non-pathogenic *Bacillus. spp.* to identify regions of the genes that are unique to the pathogen. Recent sequencing of the pX01 and pX02 plasmids (funded by this program) has identified over 200 potential genes (open reading frames) for which no function can be assigned. Using DNA hybridization and PCR assays, we are identifying those open reading frames that are conserved between B. anthracis, closely related *Bacillus spp.*, and other bacterial pathogens.

Recent Progress

Known Virulence Genes

Our first objective was to identify bacterial genes and specific regions of those genes that had homology at the DNA or amino acid level to any of five well-characterized B. *anthracis* vir genes (pag, lef, cya, capA, capB, capC). PCR primers and hybridization probes that included conserved regions of sequence were designed using homology information from database searches. Several primer pairs were designed for each target gene and tested extensively in PCR reactions for ability to amplify the target gene and potential homologs.

Dot blot and Southern blot hybridization experiments were conducted to identify gene homology between the B. *anthracis* vir genes and over 40 non-pathogenic *Bacillus* species that are potentially common in different environments. Homology between the known B. *anthracis* vir genes and multiple species of nonpathogenic *Bacilli* was detected at low stringency hybridizations. We sequenced the 16S rDNA gene of several of the positive species and conducted phylogenetic analysis to identify whether the hybridizing species were closely related to B. *anthracis*. Species hybridizing with the vir genes were widely dispersed among the *Bacilli*, indicating that these genes may be present in very divergent species. Hybriziding sequences from the other species are being cloned and sequenced to identify the extent of homology.

Environmental Testing

DNA from a wide variety of soil and other environmental samples was surveyed for the presence of B. *anthracis* vir genes using PCR. The vir gene homology study was initiated because we had observed considerable cross reaction between some B. *anthracis* specific PCR primers and a couple of environmental DNAs that we knew did not contain the pathogen. We have completed a survey of over 20 environmental samples using PCR

amplification of primers for the cap and cya genes. In this survey, we have only detected false positives between the B. anthracis genes and the native microflora in one sample, which is encouraging.

New pX01 and pX02 Open Reading Frames

We are using a combined hybridization and PCR approach to determine whether new genes on pX01 and pX02 for which no function can be assigned are specific to B. anthracis or are shared between the pathogen, other Bacilli, and other pathogenic bacterial species. PCR primers have been generated for about half of the 143 open reading frames on pX01, and each of these genes has been amplified to generate 1-kb length probes that are being used to screen DNA from a panel of 12 Bacillus spp. The hybridization assays have identified homology primarily in B. cereus 43881 and B. thuringiensis 33679 (kurstaki). PCR amplification and gene sequencing the DNA from the hybridizing species has confirmed the hybridization results and may help assign possible functions to some of these new genes. For example, gene fragments amplified from B. cereus 43881 or B. thuringiensis 33679 show 89–95% similarity to pX01. One region of similarity brackets a possible origin of replication for pX01. Experiments to determine the genomic location (plasmid or chromosome) of the homologous genes in these two species are in progress. Analysis of pX01 is complete, and we are now conducting a similar analysis of pX02 genes.

Future Outlook

We expect to complete this project in FY00. Milestones include:

- Finishing the cloning and sequence analysis of homologs to known virulence genes

- Completing comparative analysis of new pX01 and pX02 genes that have no assigned function

- Preparation and submission of manuscripts describing this work.

> Genes present on *B. anthracis* virulence plasmids are also present in nonpathogenic *Bacillus* species that are common in the environment. Investigations are under way to identify the extent of shared genes between the pathogen and nonpathogenic relatives. This work will help in design of DNA-based detection strategies that are specific for the pathogen.

Development of Pathogen DNA Fingerprinting Systems
Using Multiple Locus Variable Number Tandem Repeat Analysis

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Objective

High-resolution molecular typing of pathogens can provide (1) geographical correlation with strain types, (2) dissection of epidemics, and (3) precise genetic "matches" for forensic attribution. In order to discriminate among strains of different bacterial pathogens, we are developing multiple variable number tandem repeat (VNTR) markers. These are the most variable regions in bacterial genomes and offer the greatest discriminatory power for DNA fingerprinting. *Bacillus anthracis* is the most homogeneous bacteria described, yet VNTR markers detect great diversity among strains. When multiple VNTR loci are combined in an analysis, the potential molecular typing power is increased exponentially. The goal of this work is to develop automated and highly discriminatory markers systems in *B. anthracis* and *Yersinia pestis* for forensic and attribution applications.

Recent Progress

We have developed multiple locus VNTR analysis (MLVA) systems for *B. anthracis* and *Y. pestis* and used these systems to analyze worldwide diversity patterns. These MLVA systems use multiplexed PCR reactions and are automated through the use of fluorescent labels and standard genotyping software from Applied Biosystems. The data are standardized for easy transfer between laboratories and for database development.

Anthrax

The MLVA system for *B. anthracis* contains eight VNTR marker loci. These markers are a mixture of simple sequence repeats, complex repeats, and chromosomal and plasmid loci. These markers were developed from variable AFLP fragments or from plasmid nucleotide sequences. Fluorescent PCR primers of three different colors allow for markers of the same size to be simultaneously analyzed (see the figure on the right). In addition, amplicons vary in size such that all eight markers loci are analyzed in a single electrophoretic lane. Various combination of PCR primers have lead us to an optimum of three multiplex reactions to minimize amplifications. Automated genotyping is facilitated by "macros" that use the fluorescent color and amplicon size to identify the marker loci and alleles.

The anthrax data are being maintained in a custom "Access" database that facilitates analyses and provides a ready dissemination instrument. The Microsoft Access 97 database software

MLVA electrophoretic gel of *B. anthracis* isolates.
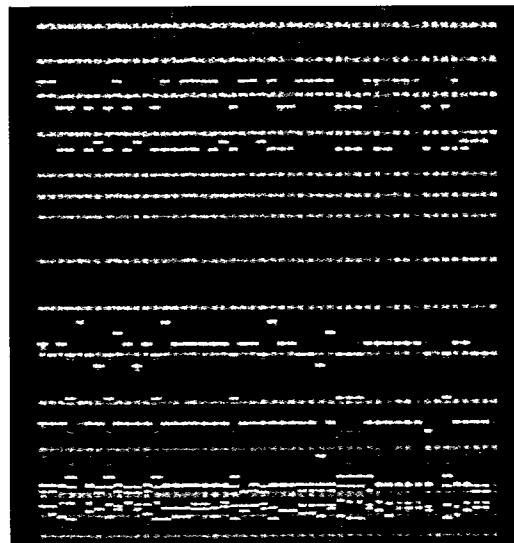
We have developed multiple locus VNTR analysis (MLVA) systems for *B. anthracis* and *Y. pestis* and used these systems to analyze worldwide diversity patterns.

is readily available to any laboratory with a personal computer. The Anthrax Genotypic Database consists of 25 items: 18 forms, 3 tables, and 4 query windows. The 18 forms also have numerous queries as intergated functions. There are 44 fields, including information about specific isolates ranging from collection site/source to genotype data to the precise location of each sample in the Keim laboratory. No prior database experience is necessary to use this database. The database is expandable to handle future samples and MLVA markers.

New VNTR markers are being discovered and tested for suitability for inclusion in the next-generation MLVA diagnostic system. Six additional VNTR loci were identified from AFLP markers and are being tested for diversity. With the development of the *B. anthracis* genome sequencing project by (TIGR), we have developed bioinformatic approaches for identifying potential VNTRs from genomic sequence. Over 800 potentially variable genomic structures have been identified, and 60 have been chosen to test for variability.

Over 400 unique isolates have been analyzed from all parts of the world, except the former Soviet Union. The world collection can be subdivided into 89 unique genotypes with the eight marker MLVA system. The genotypes cluster into approximately six major groups, probably representing clonal lineage. Particular genotypes are found to dominate major anthrax outbreak's, though the actual diversity pattern is a function of each outbreak's history. We have performed molecular epidemiological studies in North America, South Africa and Australia.

Plague
The MLVA system for *Yersinia pestis* contained 19 VNTR marker loci, as of September 1999. All of the markers are simple sequence repeats located on the chromosome (as opposed to plasmid-borne markers). The diversity of individual markers is greater, on average, than those in the *B. anthracis* system possibly due to the simple nature of the repeats. As with the *B. anthracis* system, we have developed four multiplex reactions (7, 5, 4 & 3 loci per mix) to detect variation at the 19 loci. "Macros" have been written for automated genotyping. A plague database is currently under construction, modeled after the anthrax database. We have identified over 800 additional potential VNTR loci and are screening these for utility in next-generation MLVA system.

A small set of strains representing world wide diversity has been analyzed, as well as 95 samples collected across California over the past 20 years. These genotypes are being analyzed for spatial and temporal variation patterns. We can detect the affect of geographical and temporal distance on sample relationships.

Future Outlook

Our new bioinformatic methods for identifying informative VNTRs have proven extremely effective and has eliminated one of the most problematic aspects of MLVA development: VNTR discovery. This major advancement was achieved during the FY99 period and foresees great progress in the future. Over the next grant period we will be using the novel VNTRs to fine tune the MLVA systems in B. anthracis and Y. pestis, while developing novel MLVA systems in other pathogens including Francisella tularensis.

Technology Transfer to Other Federal Agencies

In order to facilitate the transfer of the MLVA systems, we are conducting short training courses for personnel from other agencies. These consist of a one-week regime where technically skilled individuals learn wet bench procedures, instrument handling, data analysis and database interfacing. The first training course was conducted for the Centers for Disease Control and Prevention (CDC). In collaboration with our laboratory, the CDC will use the MLVA system to DNA fingerprint their entire strain collection (>1000 isolates).

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Multiple Locus Sequence Typing:
A Phylogenetic Approach for Defining Signatures

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Objective

This study utilizes comparative DNA sequencing strategies to differentiate bacterial pathogens. Multi-locus sequence typing (MLST) combines the polymerase chain reaction (PCR) and DNA sequencing to gather precise sequence information from multiple regions of the genomes of bacteria and other organisms. This information can be used to distinguish closely related species and individuals at the species and subspecies level. The method targets the analysis of common regions in related species and is therefore similar to ribosomal gene (rDNA) analysis that has been used to establish the phylogenetic relationships among organisms residing in the microbial world. MLST expands these analyses to multiple regions that evolve more quickly than rDNA to build larger datasets that greatly increases genetic resolution, particularly for close relatives that have little or no variation in their ribosomal DNA sequence. MLST sequence information from pathogens and their close relatives can now be electronically stored, transferred and shared between multiple laboratories. This technology provides a sequenced based method for comparing broad regions of the genomes of related species and provides reagents for future DNA microarray and minisequencing approaches to type and identify strains and species of pathogens (e.g., see J. Nolan, Detection Technologies).

Recent Progress

The MLST approach has been used to analyze *Bacillus anthracis* and some of its close relatives, *Bacillus thuringiensis, Bacillus cereus* and *Bacillus mycoides*. PCR primers have been designed and tested to amplify approximately 500-bp fragments from 15 distinct regions from all of these genomes. Each of these regions were then amplified and sequenced from at least 25 strains of *B. anthracis*, 15 strains of *B. cereus/B. thuringiensis* and one strain of *B. mycoides*. These analyses consistently indicate that the *B. anthracis* sequences are extremely homogeneous and that clear distinctions can be made among each of the *B. anthracis* amplicons and those of *B. cereus/B. thuringiensis*. For example, eight positions containing single nucleotide polymorphisms (SNPs) have been identified in a 466-bp region of the rpoC gene. All *B. anthracis* strains (25) are identical while one or more of these sites differ among every strain of *B. cereus/B. thuringiensis/B. mycoides* analyzed. Two general conclusions can be made from both AFLP and MLST analysis of these species: *B. anthracis* appears to have evolved from *B. cereus* and *B. thuringiensis* and there are certain strains of *B. cereus* and *B. thuringiensis* that are very closely aligned with the *B. anthracis* sequences. These SNPs combined with those from 10 other genomic regions provide a large and powerful data set that

A direct DNA sequencing approach has been used to compare 15 regions of the *Bacillus anthracis* genome to those of its closest relatives.

These studies provide reagents and a database that can be used in the forensic analysis of a specific class of *Bacilli*.

can be used to unequivocally distinguish *B. anthracis* from its closest relatives in a phylogenetically meaningful fashion.

We have begun to extend these analysis to three other pathogens: Burkholderia mallei, Clostridium botulinum and Coxiella Burnetii. Small insert libraries have been constructed form ATCC strains of Burkholderia mallei and Clostridium botulinum and clones derived from these libraries are currently being sequenced. These sequences are being utilized to generate PCR primers that can be used for DNA amplification and sequencing of target sites for MLST analysis.

Future Outlook

Single nucleotide polymorphic markers can be readily coupled to high-throughput identification assays including DNA microarrays, oligoligation assays, and other mini-sequencing methodologies. MLST analysis is a powerful tool for identifying molecular differences in closely related species and subspecies. The data generated is most useful for establishing the phylogenetic relationship among species that are difficult to distinguish by rRNA gene analysis. Current studies are aimed at developing MLST markers for at least three additional potential BW pathogens.

Publications

P. Keim, A. Klevytska, L.B. Price, J.M. Schupp, G. Zinser, R. Okinaka, K.K. Hill, P. Jackson, K.L. Smith, M.E. Hugh-Jones, "Molecular diversity in *Bacillus anthracis*.," *Journal of Applied Microbiology* 87, 215-217 (1999).

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Genbank entry accession numbers: AF065404, AF188935.

Development of a Technique Based on Insertion Sequences for the Strain Identification of *Yersinia pestis*

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Objective

We are developing a genome-wide fingerprinting method for Y. *pestis* based on the chromosomal localization of a unique insertion element, IS100. This fingerprinting method should permit unambiguous identification of Y. *pestis* at the species level and, most important, it should enable the differentiation of geographically distinct strains of this organism.

The fingerprinting method makes use of our knowledge of the DNA sequence that surround each and everyone of the IS100 elements in the genome and the ability to amplify by PCR fragments that are unique to the IS100-neighboring gene boundary. The fingerprints obtained are highly informative with respect to the genomic organization of each strain, are rapidly obtained, and are fully automatable. The resulting set of unique fragments associated with a given strain provides the fingerprint or molecular signature for that strain (see the figure below).

To maximize the information obtained by this fingerprinting we have designed two primer pairs that localize the IS100 elements in the pMT1 plasmid, one pair that localizes the single element found in pPCP and the remaining primers localize the chromosomal copies of IS100. Thus, this type of test not only makes it possible to determine the genomic variation among the tested strains but also the plasmid composition.

Data have been accumulated that indicates that a unique molecular fingerprint based on the observed diversity of IS100 distribution among Y. *pestis* enables the differentiation of strains representing almost every large geographical location in the world including all biovars of Y. *pestis*.

The fingerprinting data obtained up to now indicate that it is possible to distinguish strains according their biovar classification, plasmid composition and geographical origin. As one would expect, strains isolated from the United States give a fingerprint that is associated with the biovar Orientalis (plague is believed to have arrived to the United States from China or somewhere in Asia). Similarly, KIM, a strain originally isolated in Kurdistan and Iran and belonging to the biovar Medievalis, gives a fingerprint characteristic to this biovar. The IS-fingerprinting is amenable to multiplexing and it does not require availability of pure cultures (it works on mixed samples). Although the work performed up to

Scheme for localization of IS100 element and its utility in fingerprinting.

now has only included the use of 15 primer pairs to generate a fingerprint pattern, in the subsequent years we will expand the primer pairs utilized as well as the number of stains that will be fingerprinted. Our final goal is to accumulate a fingerprint database that contains a very large worldwide collection of Y. pestis strains and primers capable of localizing all possible positions in which the IS100 element can insert itself (regardless of strain).

The aim of this research is to develop a fingerprint methodology that will enable the unambiguous identification of *Yersinia pestis* strains and their geographical attribution. The development of DNA-based identification and detection tools for Y. *pestis* poses some important challenges. The presence of near-neighbors closely related at the genetic level, the sharing of an important virulence plasmid with related strains, and the fact that Y. *pestis* strains isolated from different geographical origin tend to contain atypical plasmids hamper the development of simple methods for its molecular identification. In addition, Y. *pestis* strains contain a large number of insertion sequence elements (ISs) scattered throughout their genomes which are highly variable with respect to their location. The methodology being developed makes use of this intrinsic variability to obtain quickly and inexpensively a "fingerprint" signature that both identifies and helps to geographically locate the origin of a given Y. *pestis* isolate. Because the method is PCR-based and ready automatable it should be readily transferable for use on specialized detectors being developed under the "Detection" section of this program located on page 65.

> **This technique makes use of intrinsic variability to obtain quickly and inexpensively a "fingerprint" signature that both identifies and helps to geographically locate the origin of a given *Y. pestis* isolate.**

Recent Progress

Using a set of 17 PCR primer pairs that localize a number of IS100 elements on the chromosome of Y. *pestis* strain CO92, we have developed a fingerprinting method that allows rapid species and stain identification of members of this group.

We have accumulated data that indicate that a unique molecular fingerprint is obtained based on the observed diversity of IS100 distribution among Y. *pestis* strains and other pathogenic *Yersinia* species.

The fingerprinting technique has been applied to a collection of some 112 distinct Y. *pestis* strains corresponding to:

- Different biovars (Orientalis, Medievalis and Antiqua)

- Distinct geographical origin (representing a worldwide distribution)

- A set having undergone multiple laboratory manipulations and passages

Typical fingerprints obtained for members of each biovar are depicted on the figure below. Multiple laboratory passages in vitro and in vivo did not change the IS100 fingerprint profile in any of the analyzed strains.

We have obtained consensus fingerprint associated with the biovar Orientalis.
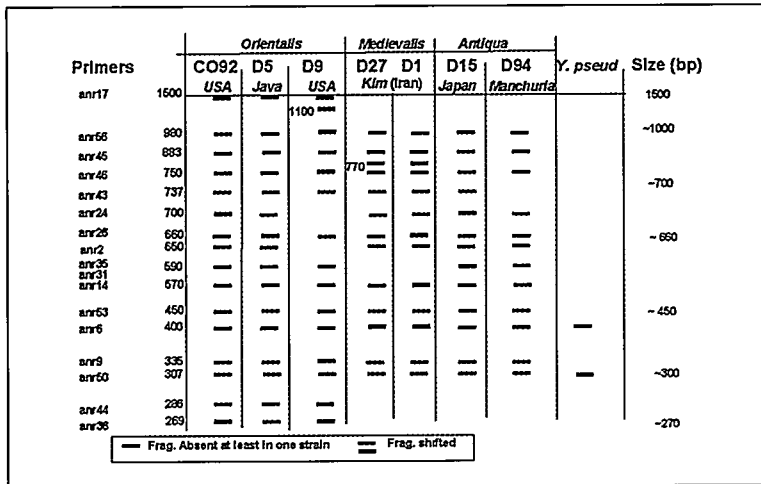
Typical IS100 fingerprint obtained with Y. pestis strains of different biovars.

Finally, in a finding that may constitute the most important development of this year, we have obtained preliminary evidence that a Manchurian strain belonging to the Antiqua biovar displays a typical pattern of the Orientalis biovar. Such a finding suggests that such "Antiqua" isolate could represent an ancestor of the Y. *pestis* clone responsible for the third (and present) plague pandemic.

Future Outlook

In the coming year we expect to get a consensus fingerprint for the biovars Antiqua and Medievalis. Such consensus will enable the unequivocal placement of Y. *pestis* strains to a given biovar and possibly to a geographical location.

We will automate our present fingerprinting methodology by using an automated fluorescent-detecting DNA sequencer (ABI377) available at our facility. Such instruments, in conjunction with its accompanying genotyping software (GenScan™ and Genotyper™) enables rapid capture of large amounts of data which can be easily manipulated and standardized. The production of standardized data from this readily available type of instrument will enable cross-laboratory comparisons and will facilitate the construction of generalized databases for pathogen signatures.

We will adapt the IS100-based fingerprinting technology for use with detection technologies being developed by the "Detection" effort of the CBNP (i.e., fluorescent micro-spheres, TacMan™, high-density arrays, etc.).

We will construct genomic libraries of each biovar representative that will enable us to localize and then catalogue each possible position on the chromosome in which the IS100 element can incorporate. With that capability we could fingerprint and cross-reference each an every Y. pestis isolate regardless of its origin

We will try to expand a collaboration with the Russian Anti-Plague Institute in Saratov, Russia, which will enable the fingerprinting of their extensive Y. pestis collection. This year we have initiated with them the sequencing of a Y. pestis-specific phage that may enable us to identify the gene(s) responsible for host-specificity and viral attachment to the Y. pestis cell. Such research could guide our effort to construct in the future non-antibody-based detectors with exquisite specificity (this phage has been shown to attach and lyse thousands of Y. pestis isolates from the Russian collection at Saratov but never a Y. pseudotuberculosis strain).

We plan to obtain additional information to corroborate our preliminary finding regarding the possible ancestor to the Orientalis biovar. Such finding would do much to elucidate the mechanisms of virulence evolution. This work will complement nicely with the whole genome comparative sequencing of Y. pseudotuberculosis, that aims to understand the evolution of Y. pestis (an obligate pathogen) from its immediate ancestor, Y. pseudotuberculosis a free-living facultative pathogen of man.

Expression Studies of Virulence Factors in *Yersinia pestis*

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Objective

The aim of this research is to identify a battery of new genes in *Yersinia pestis* that participate in the virulence process. The methodology being developed makes use of newly acquired DNA sequence from this organism to construct a high-density array or DNA chip that will enable massive and global analyses of the expression pattern of the entire genome of *Y. pestis*. Because the majority of the technology required for this project is available either commercially or through collaborators and our group and collaborators, have substantial knowledge on the biology of *Y. pestis*, the project is very likely to yield a substantial amount of new data on the virulence process in this organism.

Discovery of new virulence factors in *Y. pestis* will directly impact the development of new signatures for detection and attribution of this organism. It will also enable us to utilize the new technology to study other pathogens such as *Brucella*, for which there is substantially less knowledge of the virulence process. Our preliminary activities in the area of expression chip development has already led us to make some important contacts with scientist working on the biology of *Brucella* and *Francisella*, two organisms on which we plan to begin studies in the coming years.

Recent Progress

Isolation and Characterization of KatY

Using purified protein isolated from *Y. pestis* cells subject to temperature shift, we have characterized a new thermoregulated, chromosomally-encoded catalase-peroxidase (KatY) which is also present in *Y. pseudotuberculosis* but not in *Y. enterecolitica*. The approach employed anti-KatY monoclonal antibodies to screen a *Y. pestis* genomic library to isolate a 13 Kb insert of a positive clone. The entire insert was of sequenced and the putative peptides encoded by the open reading frames were compared with the N-terminal sequence of KatY determined by protein sequencing.

The predicted KatY gene consisted of 737 amino acid residues, possessed a prokaryotic signal sequence of 23 amino acids, and contained the motif typical of other bacterial catalase-peroxidases. Interestingly, the promoter region of the KatY contained three repeats that showed a significant homology with the consensus sequence recognized by LcrF, the transcription activator of the *Yersinia* virulence regulon. This finding is consistent with the previously described observation that KatY is synthesized by *Yersinia* during expression of the low-calcium response. The time course of the expression of KatY was determined by dot-blot hybridization with the corresponding

probes. Preliminary results showed that synthesis of katY occurred within 15 min after shift from 26° C (environment) to 37° C (host). This is the first detailed temporal analysis of synthesis of this newly-discovered virulence factor.

In preparation to constructing a DNA chip containing the entire assortment of *Y. pestis* genes, we performed a series of experiments to determine the time course of induction for a number of known virulence genes. The pattern of synthesis after induction obtained in this type of experiment is shown in the figure below.
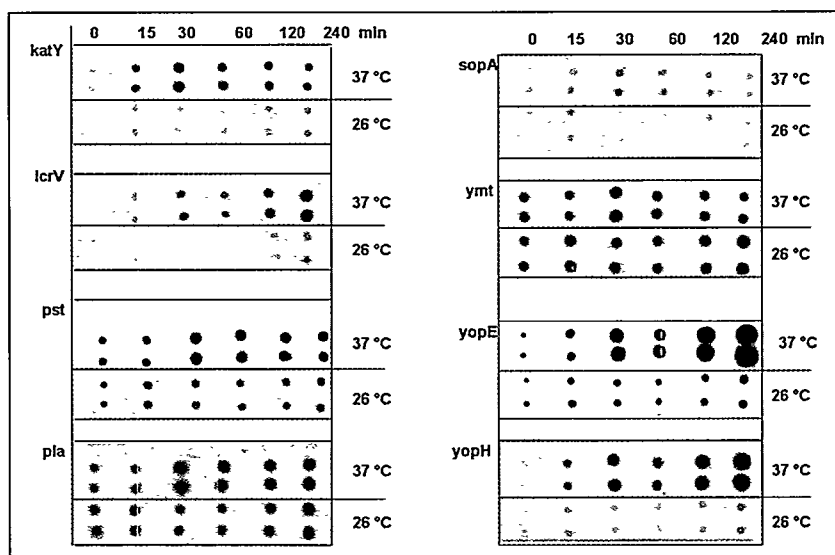
Time-course of transcriptional induction of *Y. pestis* cells after growth shift from 26°C to 37°C. A dot blot containing total RNA isolated from cells at different times after temperature shift were independently probed with DNA probes corresponding to Catalase-peroxidase (*katY*), V antigen (*lrcV*), pesticin (*pst*), plasminogen activator (*pla*), SopA (*sopA*), murin toxin(*ymt*), YopE(*yopE*), and YopH (*yopH*). The top pannel of each experiment shows the response at 37°C and the bottom that at 26°C. It can be seen that the synthesis of the catalase-peroxidase KatY is induced within 15 min. of the shift. The known thermoinducible virulence genes such as YopE ,lcrV and yopH show dramatic increases in expression within minutes of the temperature shift. Non thermally regulated genes such as plasminogen activator (pla) and murine toxin (ymt) show no change in their expression.

Future Outlook

The first task for this project next year will be optimization of conditions for deposition and detection on the proposed DNA chip. One of the major technical roadblocks for expression monitoring in bacteria is the difficulty encountered in isolating sufficient quantities of mRNA from these organisms. We will be adapting to *Y. pestis* mRNA isolation techniques developed for *E. coli* by collaborators at the University of California at Berkeley.

The actual first expression studies on Y. pestis will involve the monitoring of some 200 genes encoded in all three virulence plasmids of this organism. Such studies are of great importance because they will test our ability to monitor the expression of 50 or more known virulence genes in this organism. This work will constitute the pillar on which our subsequent work will be based since it will guide us in identifying potential new virulence genes on the basis of their co-regulation. This work will also enable us to determine the temporal expression of individual components of the type III secretion apparatus in this organism as well as all genes of unknown function encoded on the plasmids. Elucidation of the temporal functioning of this very important and ubiquitous virulence system will provide a significant contribution to the understanding of the present virulence model of Yersinia and several other pathogens that share this pathway (i.e., Pseudomonas, Salmonella, Shigella, etc).

After the work described above, we plan to perform global gene expression experiments that will include the approximately 4,500 genes encoded in the chromosome of Y. pestis. These experiments will be carried out under various physiological conditions including those encountered in organisms recovered from infected human cell lines. This type of experiment has not been possible until now and, together with experiments carried out directly from Y. pestis cells recovered from the organs of infected animals, are one of the most important experiments that will become possible from the development of this new technology. We expect to conduct the latter part of this work (FY01 and FY02) in collaboration with Russian collaborators from the Anti-Plague Institute in Saratov, Russia and/or with our collaborator at the Pasteur Institute in Paris, France.

Towards the second year of this project we plan to begin exploratory work on a similar expression chip for the BW agent Brucella. We have recently established an informal collaboration with investigators from Fullerton State University and a group in Argentina working on Brucella abortus. They have provided us with the DNA sequence for some 2,000 genes of this organism that will enable us to place them on a chip and carry out similar expression analyses. This work will allow us to begin work on a new BW pathogen while incorporating technology developed during the our studies of Yersinia pestis.

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Toxin and Virulence Factor Structure/Function Determination and Protein Signature Development

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Objective

This program provides the high resolution structural information on key protein toxins, virulence factors, and protein determinants unique to organisms or spores that are needed to enable the development of rapid, sensitive methods for the detection and identification of threat biological agents using protein-based signatures. The molecular structures of these proteins and the complexes they form with important receptors are identified using a combination of X-ray diffraction, Nuclear Magnetic Resonance spectroscopy and mass spectrometry. This information is being used to direct the design and synthesis of robust, highly specific molecular reagents that can be used in place of antibodies to detect toxins and organisms in environmental samples and exposed individuals with high fidelity and sensitivity. The primary goals of this effort are to

- Identify protein toxins and proteins produced by threat organisms that can be used to detect agent dispersal or confirm human exposures

- Crystallize and determine the molecular structure of key toxin proteins, virulence factors, and organism specific proteins

- Design suite of robust "2nd Generation" molecular reagents that bind to toxins and virulence factors with high affinity and selectivity

- Develop analogous organism specific reagents to replace antibodies and to complement DNA based detection methods

Recent Progress

Working with collaborators at USAMRIID, USAMRICD, NIH, VA Medical Center (Pittsburgh) and Walter Reed Army Hospital, we have crystallized and determined the structures of several toxins or toxin functional

Future efforts will use combinatorial methods to identify molecules that bind specifically to the surface of pathogenic bacteria and their spores for use in organism detection.

domains produced by Clostridial and Staphylococcal bacteria and complexes these proteins form with receptor molecules. Using computational docking techniques and mass spectrometry, we have also begun to identify a number of first generation molecules that bind to specific sites on the targeting (or receptor binding) domain of tetanus toxin and the Staphylococcal enterotoxin C3.

Determination of Toxin/Virulence Factor Structures

Five new toxin crystal structures were determined by X-ray diffraction: the tetanus toxin (TeNT) targeting domain (a homolog of the BoNT domains), intact botulinum neurotoxin B (BoNT/B), *Staphylococcus aureus* enterotoxin C2 (SEC2), and two *S. aureus* enterotoxin vaccine
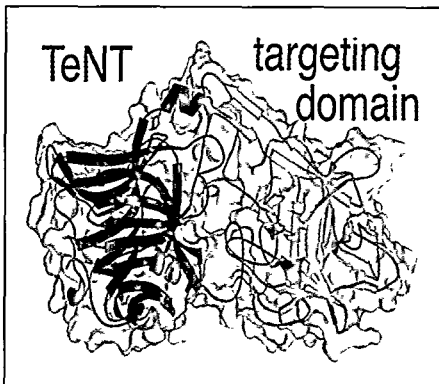
> **The information provided by these structures is being used to elucidate how the toxin binds to cell surface receptors and identify critical sites on the toxin surface that lead to toxin:receptor recognition.**
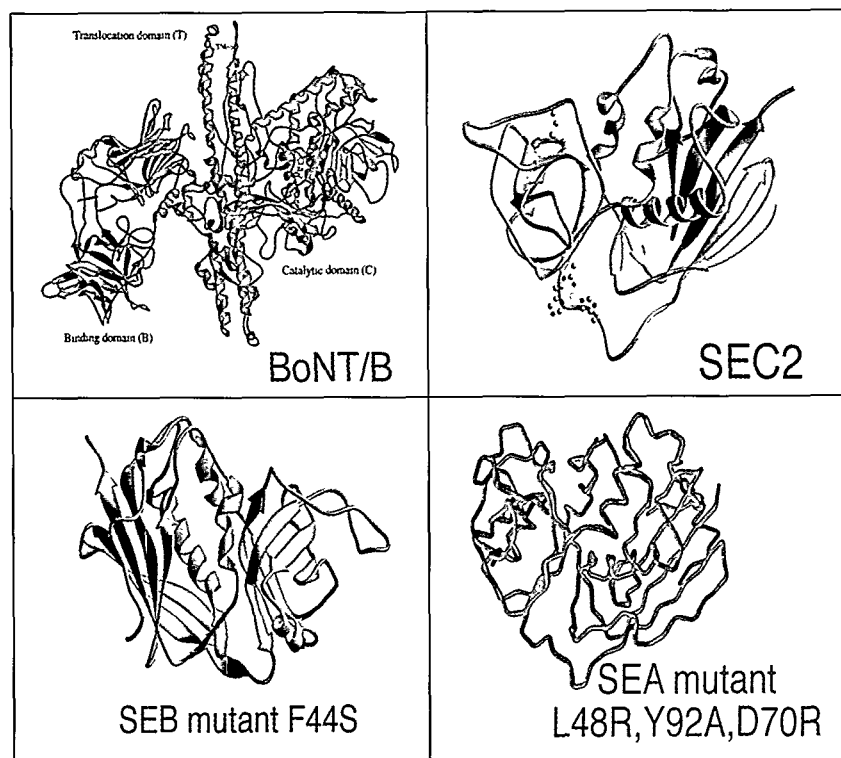
Crystal structures have been completed for the Clostridial neurotoxin BoNT/B, the Staphylococcal enterotoxin SEC2, and for two amino acid sequence variants of the Staphylococcal enterotoxins SEA and SEB. These structures have provided the information needed to begin designing replacements for antibodies used in current detection systems and for use as vaccines.

candidates, SEB mutant F44S and the SEA mutant L48R/Y92A/D70R. Three additional proteins, the catalytic domain of BoNT/A, intact BoNT/E, and NAP Hn-33, have been crystallized and current efforts are being focused on completing their structures. A virulence factor produced by *Yersinia pestis*, the V antigen, has been produced and has been screened to identify optimal crystallization conditions.

The high-resolution structure of the TeNT targeting domain (1.6Å) has been used to initiate the identification of a group of small molecules ("First Generation" reagents) that bind to specific sites on the surface of the protein using a computational technique called docking. This structure has also been used to predict the structures of a series of BoNT targeting domains by Comparative Modeling. These models, and the recently determined structures of the intact

The high-resolution structure of tetanus neurotoxin, enables the search for new molecules that bind to specific locations on the neurotoxin surface. Ligands binding to two adjacent sites will be combined to produce antibody replacements.

BoNT/A and BoNT/B toxins, are being used to identify conserved sites on BoNT for targeting the design of Clostridial neurotoxin-specific reagents.

Mode of Interaction with Receptors

Two crystal structures of the Staphylococcal enterotoxin SEB complexed with the carbohydrate portion of the ganglioside GM3 and a related sugar (lactose) were also completed. The information provided by these structures is being used to elucidate how the toxin binds to
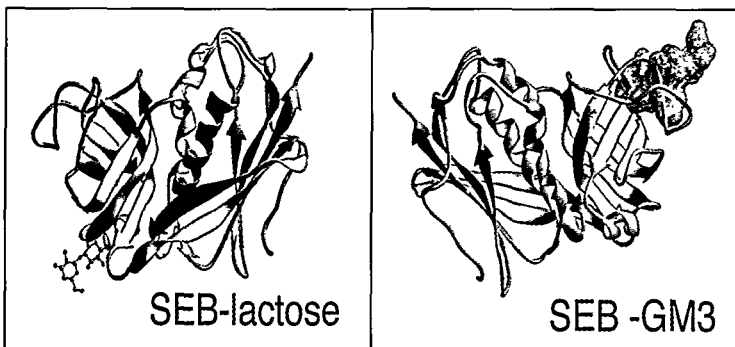
Crystal structures of the Staphyloccal enterotoxin SEB complexed with two ganglioside carbohydrates identifies the interaction sites that will be targeted when designing antibody replacements for the enterotox-

cell surface receptors and identify critical sites on the toxin surface that lead to toxin:receptor recognition. Two additional complexes, BoNT/B with a small molecule inhibitor designed by USAMRICD and BoNT/B with the VAMP peptide, were also crystallized.

Computational Modeling of Toxins, Ligand Identification by Docking, and Experimental Verification of Binding by Mass Spectrometry

Computational molecular recognition (CMR) tools are being developed to accelerate the design of new, robust molecular reagents that can replace antibodies in affinity-based toxin and organism sensors. A large database of small molecules (~240,000) is being screened by computational docking to identify new molecules that may bind to specific sites on the surfaces of protein toxins, virulence factors or other organism-specific protein determinants (such as receptors). Mass spectrometry is used to determine which of these molecules actually bind to the protein and to obtain an estimate of their binding affinity.

During the past year, a series of suitable "pockets" have been identified on the surfaces of the tetanus toxin targeting domain and the Staphylococcal enterotoxin C3 (SEC3), and sets of small molecules have been identified as possible "First Generation" ligands that might bind to these sites. Ligand sets were identified for binding to three sites on Tetanus toxin and one site on SEC3. Approximately half of the ligands have been screened for binding to two sites on the tetanus toxin targeting domain by electrospray mass spectrometry, and more than 50% were found to bind. The ligands projected to bind to Site 1, a possible ganglioside binding site, were also tested for their ability to compete with ganglioside for binding to the targeting domain using a ganglioside-liposome binding assay, and one of the seven ligands was found to compete for GT1b binding.

Development of Computational Recognition Tools

To speed up the docking step, we have designed a computational framework, DEMoS (Distributed Extensible Molecular Simulator), which will be used to perform computational docking on clusters of distributed computers. This code currently has capabilities for Molecular Dynamics simulations of proteins and ligands, and the docking tools are being incorporated. The scoring scheme used in docking is being optimized by comparing the results obtained for a group of approximately 80 protein/ligand complexes with known binding affinities. Quantum chemical methods are also being developed to treat the electronic interactions of the ligand with the surrounding solvent.

Design and Synthesis of "Second Generation" Reagents for the Detection and Identification of Threat Toxins and Organisms

By synthetically combining the best of each group of "First Generation" ligands that are identified to bind to neighboring sites on the surfaces of these proteins, we will create a new class or "Second Generation" of molecular reagents that can be used in place of antibodies for detecting toxins and other proteins with high affinity and selectivity. These

reagents will be provided to other groups to enable the development of more stable, robust detection systems for threat agents or protein signatures in exposed individuals with a substantially lower frequency of false positive signals. Our first synthetic schemes involve conjugating individual ligands to the ends of appropriate length spacer molecules to generate one bidentate molecule at a time.

Future Outlook

By the end of FY00, we will have completed high-resolution structures of the free BoNT/A light chain, and several key complexes of BoNT with molecules that identify toxin recognition mechanisms and regions of the molecule required for receptor/substrate specificity. Work will be nearing completion on the solution of the complete structure of the final BoNT serotype, BoNT/E. Future work will focus on obtaining high resolution structures for the other serotype targeting domains to complete the Clostridial neurotoxin structures needed to identify conserved structural sites and to design the BoNT-specific Second Generation reagents. The structure of the V antigen of *Y. pestis* and edema factor of *B. anthracis* will be completed by early FY01, and efforts will turn to identifying and characterizing the structures of key bacterial and spore protein determinants that will aid the detection effort.

We will implement the boundary element method (BEM) for solvation into the Massively Parallel Quantum Chemistry code (MPQC), and algorithms using flexible docking will be compared with the regular docking procedure to determine if they provide a better prediction of the most stable binding sites in our docking studies. Scoring schemes used in the docking algorithms will also continue to be evaluated and improved using sets of know protein-ligand interactions.

The next computer modeling and docking studies will focus on the BoNT targeting and catalytic domains, using the coordinates provided by the structural studies to identify structural "pockets" and sets of ligands that might bind to those sites that appear to be conserved and essential (e.g., they cannot be altered by genetic engineering). Following the synthesis of the prototype bidentate reagent for TeNT detection to confirm the utility of the approach, an efficient combinatorial synthesis scheme will be developed to generate suites of these Second Generation reagents for detecting the BoNTs. This approach will then be extended to develop similar reagents for the Staphylococcal enterotoxins, ricin, protein components of anthrax toxin (for use in detecting/confirming anthrax infection), and other protein determinants produced by threat organisms.

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Signature Pattern Development for Detection of "Out-of-Place" Organisms

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Objectives

BW agents possess distinct molecular features that can be used to monitor their natural occurrence in the environment. In order to distinguish a natural instance of an organism from a subtly modified, unnatural variant with enhanced virulence, we need (1) a molecular "signature" to characterize the natural organism's background variation; and (2) a sensitive statistical test to recognize variants that differ significantly from this background.

In this project, we have focused on approaches to identify highly informative molecular signatures, using DNA and amino acid sequence data from viral organisms. Our recent goals were:

- Compare the statistical characteristics of three methods for defining signature patterns.

- Examine the sensitivity of the methods to sample sizes.

Recent Progress

After our focus on hepatitis C earlier in this project, we moved to influenza as a model organism. Influenza has many characteristics of a potential BW agent: it is highly contagious and is spread as an aerosol; it can be very lethal, with death rates, such as during the 1918 pandemic, that far exceed any seen due to other diseases in the 20th century; a vaccine can be produced for protection of a rogue nation against a modified strain, but it takes too long to produce vaccine to protect a victimized nation; a few subtle changes, possibly as few as one amino acid change, can lead to dramatic changes in its virulence; several hemorrhagic fevers of BW concern, such as Lassa, Junin and Machupo, possess similar genomic structures and are also spread as aerosols; and epidemics caused by influenza bear the characteristics of rapid, wide spread that one might expect from release of an aerosol, contagious BW agent.

The genes of influenza mutate at a rapid rate. This variability makes it feasible, and efficient, to develop methods for detection of "out-of-place" influenza viruses on the basis of genomic sequence data.

> Influenza is highly contagious and spread as an aerosol; it can be very lethal, with death rates that far exceed any seen due to other diseases in the 20th century.
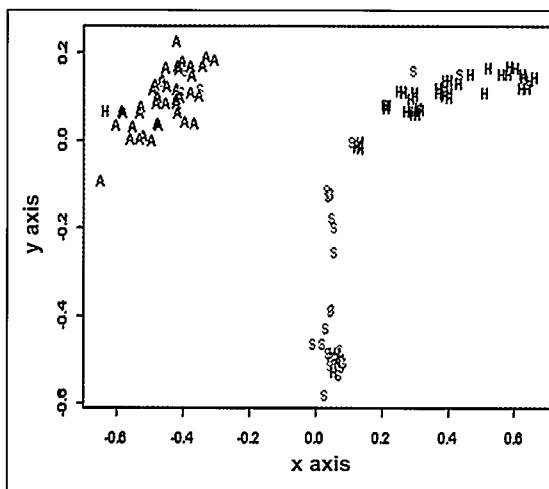
Techniques for Pattern Recognition

We have followed two basic directions toward signature pattern development: supervised learning, and unsupervised learning. Supervised learning uses data from known classifications (the "training set") to identify characteristics in the data that both strongly indicate class membership, and accurately distinguish among classes. We considered two methods of supervised learning: VESPA and principal components analysis (PCA). The latter method is standard in the literature for quantitative characteristics; our contribution was to extend its use to categorical data. VESPA was developed in earlier years' efforts under this program

Unsupervised learning assumes no prior knowledge of classifications. A distinguishing pattern must be determined by examining data in different ways, in search of a view that clearly delineates meaningful groupings. The unsupervised technique that we focused on was phylogenetic tree reconstruction, which depends upon a model of the evolutionary behavior of the influenza virus.

Supervised Learning

Principal components analysis (PCA) attempts to define two or a small number of orthogonal axes that capture as much of the variability in the sample as possible, such that the predefined classes overlap as little as possible. In the figure below, sequences from avian, human and swine hosts are plotted on the axes given by the first two principal components. To generate the figure below, genetic distances among sequences were estimated under a simple model of evolution. Samples were then plotted on (x,y) coordinates such that Euclidean distances among the sequences were as close as possible to the corresponding genetic distances, thus achieving a huge reduction in the dimensionality of the problem.

These two axes allow a clean clustering of sequences by host, with 14 cases out of 129 being misclassified. Upon researching the sources of the misclassified cases, we learned that all were instances of cross-species transmission, and that the

Principal component analysis of influenza genomic sequences shows clustering by host. (A = avian, H = human, S = swine)

host of origin belonged to the class with which it was clustered. For example, the "human" sequence in the "avian" class was taken from a person infected from poultry.

By exploiting the derived functional relationship between genetic distance and (x,y) coordinates, we can place a novel sequence on this map, and assign it to a class with a high degree of certainty.

The figure below illustrates PCA applied to human influenza sequences from a 30-year time period. Our goal here is to determine a genetic signature of "place" in time. While it is difficult with this amount of data to distinguish years, it is clear that sequences can be clustered (somewhat crudely) by decades.
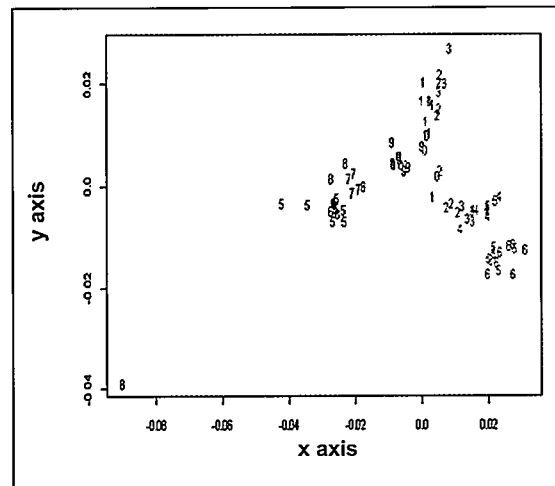
VESPA is not illustrated here, since this work was described in earlier reports. When applied to the dataset for PCA above, the classification results were exactly the same.

Principal component analysis of 30 years of human influenza genomic sequences shows temporal clustering by decades. (Digit = year, Black = 1960s, Red = 1980s, Green = 1990s)

Unsupervised Learning

The techniques described above share attributes of simplicity, and they share the detraction of falsely assuming that all sequences are independent. Thus, these techniques are susceptible to vagaries of a possibly nonrepresentative sample. Phylogenetic tree estimation is an important fundamental technique, designed to capture relationships by evolutionary descent among nucleotide or amino acid sequences. We used phylogenetic trees to classify sequences by "clade" or distinct lineage. Sequences that are members of a clade are more closely related genetically to each other than to sequences from other clades.

To estimate a phylogenetic tree, one needs a model of the evolution of the organism. We explored a hierarchy of complexity of models of evolution, using a sample of 80 human influenza viral sequences from the years 1968–1995. While details of the branch lengths of the tree were sensitive to the model, clustering of sequences changed little between models of moderate complexity and models of maximum complexity. Because computational time increases dramatically with complexity, we

based our development on models of evolution with moderate complexity, namely the F84 model with site-specific rates of evolution. We applied this mode of evolution to sequences from 1992–1996, with an additional sequence from 1968, the date at which the viral lineage purportedly arose. The results are given in the figure below. The sequences from 1992–1996 are color-coded by year. It is clear that significant overlap of lineages exist, indicating the need for more data in order to improve the power of discrimination.
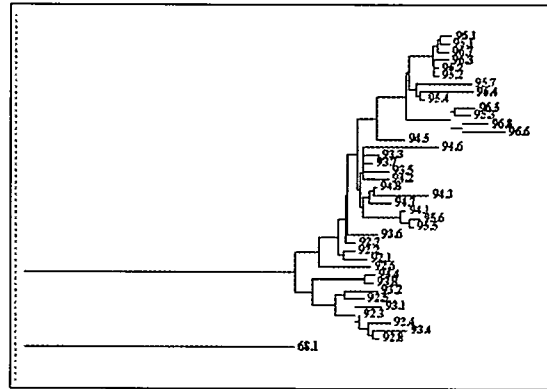
Phylogenetic tree showing the evolution of human influenza virus. (Influenza Hemagglutinin, 1968, 1992–1996)

Which Technique?

For the datasets considered here, all techniques had a similar power to correctly assign samples to classes. However, we were operating under fortunate circumstances of ready access to large datasets, so that we could cull sequences and achieve even representation over time and within classes. We also could predefine a training set. In practice, these two predisposing circumstances may not be in place. It is our opinion that approaches to classification or detection of "out-of-place" events should be based on phylogenetic tree estimation, to reduce sensitivity to nonrepresentative data sets. The disadvantage of these techniques is their computational intensity, a disadvantage that is rapidly diminishing as supercomputers become readily accessible.

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Cooperative Epidemiology: The Nexus of Biological Weapons Proliferation and Emerging Diseases

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Objectives

Under the CBNP in FY98-99, a one year pilot-scale regional monitoring network that incorporates advanced telecommunications technology was carried out in Chelyabinsk, Russia in cooperation with hospitals in New Mexico to ascertain the prevalence of and risk factors for acquisition of Hepatitis C. In this follow-on work, we are investigating the same disease in Russia (near Novosibirsk) and in northern Kazhakstan (near Stepnogorsk). The project will exercise epidemiological tools, information collation and analysis, and information distribution, which could be of great value in monitoring for the Biological Weapons Convention (BWC). We seek to increase the ability of regional public health departments in the former Soviet Union (FSU) to provide clinical expertise in identifying and reporting the rapidly changing patterns of this disease.

Background

The spread of emerging diseases constitutes an international public health emergency. Understanding the origin, clinical effects, and prevalence of these novel or recurring diseases is essential for planning intervention strategies. At the same time, unusual disease outbreaks may represent evidence of biological weapons proliferation; investigation of such outbreaks could increase confidence that such episodes arise from natural events, as well as develop the cooperative means for addressing concerns of biological weapons proliferation around the world.

As mandated by the President, under current BWC negotiations, the U.S. will seek to strengthen existing confidence building measures (CBMs) and to negotiate on-site inspections under two specific conditions:

- Suspicious outbreaks of disease.
- Allegations of biological weapons use.

Further, the U.S. has sought to open Russian biological weapons laboratories to international scrutiny. The current phase of the Hepatitis C project will take place with the State Center for Virologic Research in Novosibirsk,

The morbidity and mortality from "emerging diseases" is enormous - Hepatitis C alone affects over 3 million Americans, and untold millions more around the world.

Russia (also known as VECTOR) and the National Center for Biotechnology of Kazhakstan (NCB-RK) in Stepnogorsk, Kazhakstan.

In order to address the President's mandates and to provide a near-term tool to meet the needs of the legally binding regime under negotiation, a networking tool for exchange of information is desperately needed. Current CBMs are filed by less than 20% of signatories to the BWC, and those that are filed are in paper format, which are largely unusable by States Parties.

Emergency room and clinic populations will be utilized for this project, as they provide populations which are largely unselected, a problem common to previous Hepatitis C surveys that have focused on known risk groups. Our experience from Chelyabinsk-70 suggests that there are novel risk groups, although the data are still being analyzed. This information has been factored into the risk-factor questionnaire being developed for this portion of the project.

Approximately 2000 volunteer patients over the age of 18 from the Russian and Kazhak sites will have serological studies for Hepatitis C performed. They will also respond to a detailed questionnaire of known and possible risk factors for Hepatitis C, designed with the assistance of the World Health Organization and the University of New Mexico School of Medicine. All data will be entered by participating centers. Serology will be performed using standardized test kits at each of the sites. In addition, laboratories will maintain serum samples for possible later analysis of genomic structure of Hepatitis C isolates. All data will be transmitted over the Internet to a central server at Sandia National Laboratories and will be accessible to all parties at all times.

The final phase of this work will be to publish the results in an internationally recognized epidemiology journal. Data will be shared with the international negotiating community in Geneva. All data will be authenticated and patient confidentiality protected. Those patients with positive results will be provided counseling for mitigation of Hepatitis C disease progression.

Recent Progress

Data gathering was completed from approximately 2000 patients in the Chelyabinsk region and from roughly 1200 emergency room patients in three hospitals in New Mexico. This study is almost certainly the largest of its kind ever performed, providing a large amount of data on known and potential risk factors for acquisition of Hepatitis C.

The Cooperative Epidemiology project has demonstrated that complex, novel diseases can be investigated by non-specialist physicians and provide information of importance to health care providers, while establishing a network of data gathering that could be exploited to resolve allegations of biological weapons use or development.

Although data analysis is still preliminary, we have learned the following:

- The prevalence of Hepatitis C is much higher in the central Urals region of Russia than expected.
- It appears that ingestion of potential hepato-toxins (certain medications and also alcohol) is associated with Hepatitis C.
- Surgery, but not necessarily blood transfusions, is associated with Hepatitis C acquisition in Russia.

Russian and Kazhak biological weapons laboratories in both the civilian and military sector have submitted research proposals similar to this work. We believe that this is an important milestone in the conversion of these institutes to legitimate purposes.

Next steps

We anticipate developing similar surveys for real-time outbreak monitoring of acute respiratory illness, beginning with influenza and evolving to include significant pediatric infectious disease as well as novel hemorrhagic fever syndromes.

In addition, we will:

- Develop project plans for real-time monitoring of influenza epidemics in northeastern Asia. It is highly likely that new strains (or variants) of influenza arise each year in this region of the world. This is critical information for development of annual vaccination strategies.

- Negotiate with the Kirov biological weapons laboratory for similar work. If we are successful, this will be the first time that a military bio-weapons site will engage in collaborative research with U.S. laboratories.

- Seek additional funding from the Centers for Disease Control and Prevention for permanent laboratory and clinical disease surveys at multiple FSU biological and public health laboratories.

Improving Predictions of CB Agent Behavior in Buildings

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Objectives

Buildings represent a key element in understanding the potential consequences of an urban release of chemical or biological (CB) agents. Most people spend about 90% of their time indoors—in homes, places of employment (e.g., offices), and public buildings (e.g., airports, shopping malls). Buildings and their occupants may be either direct targets for an agent release, such as a release into a heating/cooling/air conditioning (HVAC) system or from within the building itself, or indirect targets as a consequence of being downwind from an outdoor release.

As a result, building interiors represent a very large exposure potential for individuals or for collective populations. Contaminants behave differently indoors than outdoors. Air flow and contaminant transport is driven by mechanical (HVAC) systems and/or by the interaction of the building with the external environment. Building interiors also contain many surfaces onto which potential agents may deposit and from which they may be released, leading to the potential of secondary contamination. Buildings may also provide protection in some circumstances, so-called "shelter-in-place".

This project seeks to improve our ability to estimate exposures to various CB agents under a variety of release scenarios and building conditions. In addition, a better understanding of the transport and behavior of agents into and within buildings will improve the design and implementation of mitigation methods and will benefit the design, deployment, and (ultimately) the interpretation of signals from detection systems.

Recent Progress

Our research during the past year has focused on two main topics:

- the behavior of aerosols
- the development of a series of prototypical buildings as a means of examining agent exposures indoors

Exposures most biological agents result from inhalation of aerosols. While aerosols have been the subject of considerable research, relatively little is known about the transport and fate of the larger bioaerosols. We are currently investigating the deposition losses of large aerosols (particles with diameters larger than 1 micrometer) in three systems:

- within rooms under a variety of air flow conditions
- in HVAC systems
- in leakage pathways in the building shell (these pathways account for much of the air infiltration into a building)

This project is aimed at providing high-fidelity estimates of exposures to Chemical/Biological agents for First Responders and building managers.

Modeling CB behavior in building interiors is important. People spend as much as 90% of their time inside buildings.

First Responders, typically fire or police agencies, need to know what responses are appropriate based on the quality and quantity of information they have available.

Within rooms, deposition losses increase significantly when air motion within the room (often referred to as turbulence) increases. We have improved our indoor aerosol models to account for results we have obtained in laboratory experiments. There are surprisingly few data on aerosol deposition in building HVAC systems. Our preliminary modeling has suggested that even without explicit aerosol filtration, aerosol losses in duct systems may range from 40% to over 90%. In order to verify and improve our models, we have completed construction of an experimental HVAC system that will enable us to study these losses in great detail. Similarly, there are few data on aerosol transport through building leaks. Our current models and experiments, based on flows through simple crack systems with smooth walls, show minimal losses for aerosols less than a few micrometers in diameter but a rapid increase in losses for larger aerosols.

The initial focus of our prototypical buildings research has been to model intermediate-size office buildings with a goal of examining a number of exposure scenarios and the influence of various underlying parameters. One immediate objective of this work is to identify possible "rules of thumb" for first responders. Examples of such guidelines might be: Where in the building might exposures be highest (and thus deserving of rapid attention)? Can HVAC systems be turned off or otherwise manipulated to reduce exposures in all or part of the building? The assembly of building characterization data and the exposure modeling have been completed, and the analyses of the simulation results have just begun.

Future Outlook

The overall goal of this work is to provide high-fidelity predictions of exposures to CB agents within buildings. Our current multizone air flow model (COMIS) and aerosol behavior models will form the basis for an experimentally evaluated set of models for integration into CB-ARAC. As with the aerosol studies described above, we will also examine deposition and reemission of gas-phase chemical species. This work will include the development and testing of a physico-chemical model for sorption and desorption that incorporates the properties of specific agents. The recently initiated collaboration between the DOE laboratories and the DoD facilities at Edgewood will provide critical information.

A number of improvements and additions to our air flow models are planned, including the use of an experimentally tested computational fluid dynamics model for air flows within large single rooms. Finally, we will increase our interactions with various first responder agencies to ensure that the guidance developed from this project is relevant to their needs.

Field Tests

In conjunction with an EPA- and DOE-funded project to investigate indoor aerosol exposures, we have begun a series of controlled experiments at field sites to study the penetration and removal of aerosols from buildings. This work has begun with establishment of a test house at the University of California Field Research Station. Later work will involve buildings located in Fresno, California, as part of the San Joaquin Valley study.

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Building managers or owners, interested in methods to detect agents and reduce potential exposures, need to know what building mitigation strategies are available and appropriate for their situation.

Modeling and Prediction

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Modeling and Prediction

Modeling Chemical/Biological Dispersion in Subway Systems

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Objectives

The objective of the subway system transport and fate work is to develop a validated suite of models that can predict the time-dependent history of flows and concentrations resulting from a chemical/biological (CB) release in a subway system. Releases can occur in a subway station, down a vent shaft, on a train, or from the outside of a moving train. The capability being developed includes complex models containing the important physics used for preplanning operations and rapid-running codes linked to sensor arrays for emergency response in subway control centers. These codes can assist in preparation of subway vulnerability analyses, determination of emergency response strategies (e.g., ventilate or contain), evaluation of engineering mitigation strategies, hazard and contamination assessment during an event, training emergency response personnel, and forensics in a post-event reconstruction.

Recent Progress

The CB extension to the Subway Environment Simulation (SES) model is currently carried out through a post-processor to SES Version 4.0. This code predicts one-dimensional time histories of velocity, temperature, and relative humidity for each subsegment of a subway system.

The Argonne CB agent dispersion model has been enhanced in the following ways:

- The inclusion of particle deposition due to sedimentation, forced convection, and thermophoresis
- The addition of transport due to uptake and discharge of agent from traincars
- The visualization of the dispersion model output in an animated form that illustrates the spread of agent both below and above ground. Deposition onto tunnel and station walls is currently treated.

Our treatment of the train car in the CB dispersion process recognizes that train cars will uptake agent as they pass through the plume and then reemit agent as they travel throughout the subway system. The uptake and reemission is due to the exchange of train car air with air in the tunnels and stations. The air exchange rate depends on the operating characteristics of the traincar HVAC system, the length of time the doors are open at the stations, and the flow of passengers onto and off of the trains at the stations. Higher train car exchange rates lead to more uptake of agent into the train car and quicker reemission of the agent once in the car.

Chemical and biological agents released in a subway system are spread by train movement and affect people both below and above ground.

Application of the SES model to a generic subway system in the category of "more recently built" subways has led to the following findings for the cases analyzed:

- Transport due to the piston effect of the train motion needs to be supplemented by the uptake and reemission of agent from traincars to obtain a correct picture of the dispersion in a subway system
- Agent typically first arrives at a station due to transport by traincars (unless the release is in that station)
- A significant fraction of the particles in the 3- to 5- micron range were ventilated to street level where they could become an above-ground hazard.

An entire bioincident can last 2 to 3 hours within the subway system if no action is taken to stop trains.

An animated version of the dispersion model output was developed for both scientific and illustrative purposes for subway clients. The simulation (see figure below) shows a frame of that video illustrating the spread both below and above ground from an in-subway release. The movie shows the number of lives that can be saved by stopping trains immediately after detection.

Data from releases of a biological simulant in 1966 in the New York City subway system are being used to help develop better understanding and better computer models of agent spread in a subway.

Finally, work on testing the model with the 1966 biosimulant releases in the New York City subway system was initiated. In these tests, releases of a biosimulant were made from moving trains and down vents into subway stations in Manhattan. Measurements were made over a variety of time periods at fixed points at nearby stations and with mobile instrumentation simulating the motion of passengers through the system. Test I of five tests was used for model/data comparisons. This comparison showed that the SES model correctly represents the general behavior in the data, although a quantitative comparison requires theoretical improvements to the model.
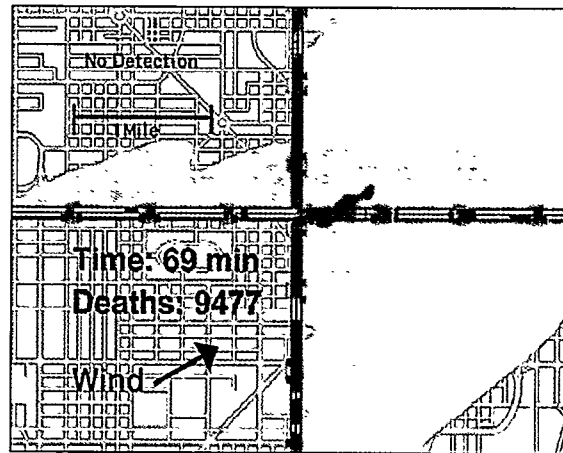
Computer-predicted spread of a biological agent above and below ground from a release in a subway system.

Future Outlook

Future work will combine field and laboratory data with improvements in the theory. The causes of model/data discrepancies will help direct the order of priorities in the ongoing model improvement. The effort to improve the station modeling will involve laboratory studies to understand the general flow patterns in stations, emissions to street level, and proper proportion of agent staying underground versus being ventilated above ground. CB agent physics will also be added to the SES model. Finally, a fast-running version will be developed to be installed with detectors in a subway to assist the prediction of above- and below-ground effects during an incident.

Field Tests

Smoke releases and measurements were made in a subway station to better understand the spread of chemical and biological agents (see the PROTECT summary in the DDAPs section).

Building-Scale Exterior Modeling

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Objective

The objective of this effort is to develop validated modeling capabilities for the transport and dispersion of chemical and biological (CB) agents released near buildings in an urban environment. These capabilities are achieved via sophisticated computational fluid dynamics (CFD) models, with appropriate physics for nontime-critical applications and a simple but computationally fast analytical/empirical model for immediate response applications. These capabilities can be used, for example, to track CB dispersion patterns to help first responders with emergency planning of special events, to perform vulnerability analysis and to support post-release analysis. A longer-term goal is to integrate these capabilities into CB-ARAC.

Recent Progress

The LLNL and LANL efforts in this area were concentrated on improving the model physics and numerics, model validation studies using laboratory and field experiments, and simulations for flow and dispersion around a specific building. Significant model enhancements were accomplished for the FEM3MP and HIGRAD models, the former designed primarily for modeling a square area of 1 to 10 km, and the latter for an area of 10 to 100 km in size.

Model Development

Progress in the model development areas for the CFD models has been focused on extending the capabilities of the existing models to CB relevant applications. The following enhancements have been implemented and tested during this year:

- Aerosol effects, including dry deposition and gravitational settling.

- First-order ultraviolet decay for biological agents.

- Surface energy budget.

- Shading and tree canopy effects.

- An advanced, nonlinear eddy viscosity, Reynolds-averaged (RANS), and several large eddy simulation (LES) turbulence submodels.

- Massively parallel version of the models on the ASCI platform.

- Improved numerics including the implementation of conjugate gradient and multigrid solvers.

- Improved mesh generation capability using urban databases.

These new physics and numerics enhancements allow increasing realism in the representation of the dispersion scenarios of interest and greatly increase the types of agents that can be simulated.

Model Validation

CBNP has sponsored the Fluid Modeling Facility to conduct laboratory experiments at the NOAA/EPA wind tunnel for the purpose of providing comprehensive datasets to validate the CFD models. These experiments are focused on the physical modeling of flow and dispersion of releases within multiple block arrangements. The initial experiments are based on a 7-row, two-dimensional, street canyon configuration. The following figures on the right show the experimental data in comparison with preliminary results from the CFD models using the RANS and LES turbulence submodels.
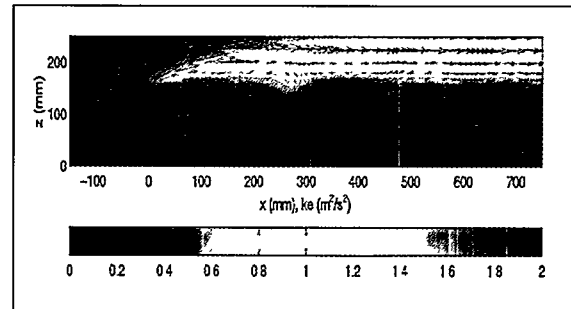
The preliminary results from the two submodels have produced reasonable agreement of the mean velocity and turbulent kinetic energy fields with the steady-state wind-tunnel flow data. Major features, such as the flow separation and recirculation at the upwind edge of the first
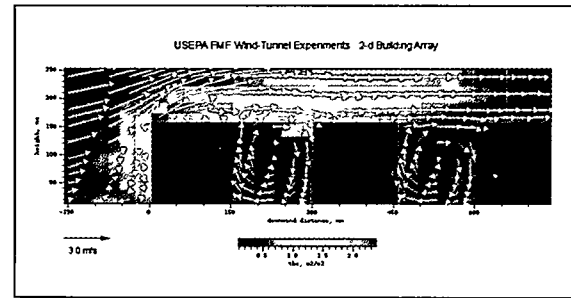
Mean wind vectors and turbulent kinetic energy (TKE) field measured along centerline around the 2D block array in the NOAA/EPA wind tunnel. The first three of seven 2D blocks are shown.
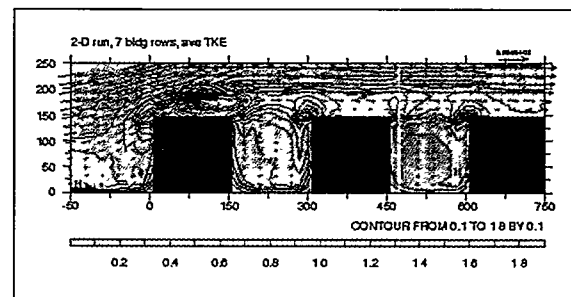
LES modeled mean wind vectors and TKE field. The first three blocks are shown.

RANS modeled wind vectors and TKE field. The first three blocks are shown.

The FEM3MP and HIGRAD models have been greatly enhanced in physics and numerics, and are now running on the ASCI massively parallel computing platform. These enhancements will enable simulations of the flow field and dispersion patterns of CB agent releases to be performed with more accuracy and much higher computational efficiency.

Modeling and Prediction

block and clockwise vortices in the canyons, are reasonably well reproduced. The RANS model was able to yield results for the mean fields at far less computational cost than the corresponding LES models; however, the latter appear to capture the transient development of the vortices more accurately.
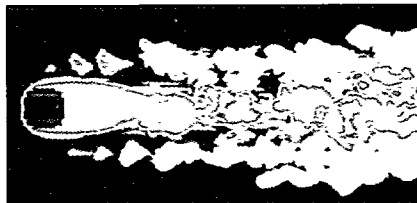
Model Applications

As part of the CBNP program, both LANL and LLNL scientists have selected high-probability target areas (e.g., Washington DC Mall area) at which to perform high-fidelity tracer dispersion simulations. Recently, a collaboration with the BASIS team at LLNL and LANL was established to model specifi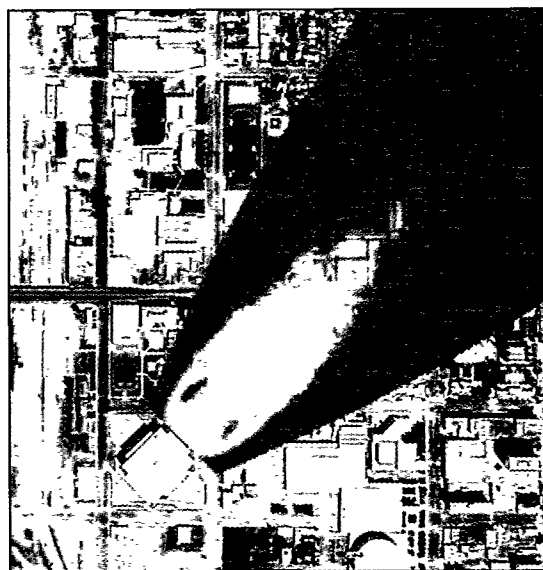c buildings in selected urban areas. The figure on the right shows the instantaneous concentration from a simulated agent release inside a large building with the material being vented to the atmosphere through 16 elevated


A large-eddy simulation model computation of the instantaneous tracer transport around an isolated building showing the turbulent nature of dispersion (concentrations at a height of 5 m and 10 minutes after the release start time).

HVAC ventilation ducts. Indoor dispersion has not been modeled; instead, the focus was on the downwind hazard posed by the venting of the toxin. Using a LES approach, it is possible to simulate the resolved-scale instantaneous concentration fluctuations and the time-averaged dispersion properties. The results of this simulation are being used to characterize the source term for the BASIS puff dispersion model and to aid in biological agent sensor placement around the building.

For this demonstration, the near-field CFD-generated dispersion pattern is used as the source term for a BASIS puff dispersion calculation (figure at right) that generated a plume with two high-concentration "eyes." These "eyes" reflect the vortex circulations that have been captured by the high-fidelity CFD approach and used in the char-

Tracer dispersion pattern from the BASIS puff dispersion model for an elevated release showing significant differences when using results from a CFD-generated near-field source term accounting for building effects.

Preliminary results from this model validation study using data from one of the DOE-sponsored NOAA/EPA wind-tunnel experiments indicate that the FEM3MP and HIGRAD models are reproducing the results measured in the wind tunnel. The LES model was able to yield time-varying results more accurately; the RANS model was able to produce comparable results for the mean fields at far less computational cost.

Initial simulations around a large urban building clearly suggest that the ground-level concentration and dosage patterns are distinctly non-Gaussian.
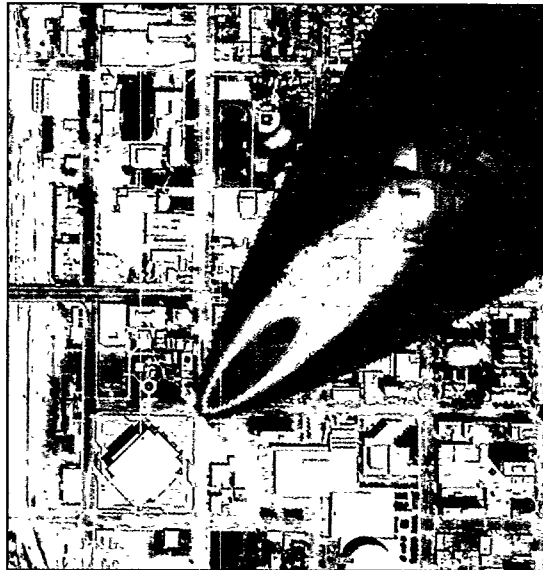
Tracer dispersion pattern from the BASIS puff dispersion model for an elevated release showing significant differences when using results from a traditional point-source term.

acterization of the source. A similar BASIS puff dispersion calculation (figure at left) with a traditional Gaussian puff source term did not exhibit this intricate dispersion pattern but, instead, depicted the normal Gaussian-shaped plume. The present calculations considered one isolated building. Future calculations will model a much larger area that includes several hundred buildings around the site of interest.

Future Outlook

The past and current exterior buildings dispersion effort has been largely focused on the development of the modeling tools required to perform simulations. The basic framework of most of these tools has now been developed and future work will emphasize intense testing, evaluation and application of these new tools. Validation of the models will continue on new data from the NOAA/EPA wind tunnel experiments and on a series of LLNL on-site field experiment. The models will be applied to the Salt Lake City area in coordination with field experiments that will be carried out in October 2000 under DOE's Vertical Transport and Mixing (VTMX) program and supplemented by CBNP's field experiment effort. The goal of this exercise is to develop and test a multiscale prediction capability whereby regional forecasts from the NRL/LLNL COAMPS model will provide the up-to-date meteorology to drive a series of higher resolution calculations from HIGRAD and FEM3MP down to building-scale levels. Such a combination of COAMPS, HIGRAD, and COAMPS will allow for unprecedented transport and dispersion calculations ranging from regional, to urban, to building scales.

Regional-Scale Exterior Modeling

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Objectives

The objective of the regional-scale exterior modeling task within the CBNP Modeling and Prediction thrust is to develop regional atmospheric models for accurately assessing the larger-scale CB agent plume transport (10s to 100s of kilometers) resulting from terrorist attacks in urban environments. We have identified two important areas in which regional atmospheric models are deficient for these specific applications:

- Quantification of the uncertainty in the computed transport winds and other meteorological variables.

- Lack of building influence on the wind, temperature, and turbulence fields.

We are developing methods for understanding the uncertainty in model predictions and the range of possible outcomes through ensemble forecasts so that we will be able to estimate, with some measure of certainty, the regions that are impacted by the toxic plume. We are also devising parameterizations to account for the drag, turbulence production, and shading induced by buildings so that the transport and dispersion of CB agents within cities is more realistic.

Recent Progress

Dispersion Forecast Uncertainty

We are working on an approach for quantifying meteorological uncertainty via development of an ensemble of forecasts from slightly perturbed initial conditions to predict the time evolution of the probability density function of atmospheric variables. In other words, we are developing methods for understanding the uncertainty in model predictions and the range of possible outcomes.

Meteorological forecasts, which are used to estimate where a CB agent plume will travel, contain uncertainty due to both errors in the input data and from errors in the model. We are using ensemble forecasts (i.e., making several forecasts for the same time period by varying the initial conditions) to estimate the range of possible outcomes so that we can better estimate the regions impacted by the toxic plume. For the case illustrated in the figure to the right, we are using a 48-hour forecast from COAMPS (Coupled Ocean/Atmosphere Mesoscale Prediction System, originally developed by the Naval Research Laboratory) to drive the Lagrangian dispersion model LODI. The results from a simulated release for hour 33 are shown, where the center of the domain is at the Oakland airport and the basic on-shore wind is from the southwest. In three of the ensemble
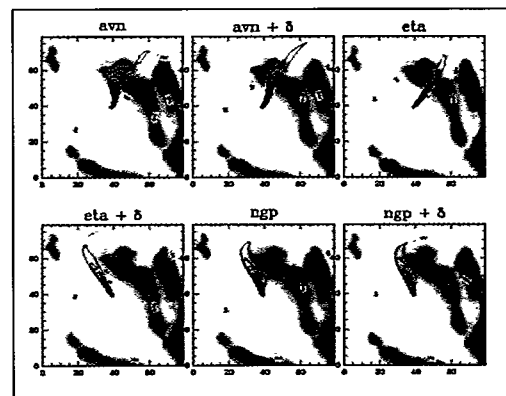
Concentration contours from the LODI dispersion model showing the effect that small variations in initial meteorological conditions can have on the resulting contamination.

The COAMPS meteorological model was used to create an ensemble forecast. The different initial conditions were created by using different large-scale models to initialize COAMPS and by randomly perturbing those initial conditions. The resulting winds are used to drive the LODI dispersion model, producing concentration contours that are overlaid on the terrain.

The regional-scale model will be used in conjunction with models being developed in the other Modeling and Prediction areas to simulate the transport and fate of chemical and biological agent releases from the building and subway interiors out to building exteriors and on up to the many-building and regional scales.

forecast simulations, the plume goes over the top of the hills to the northeast; in the other three simulations, the plume hugs the coastline and goes around the hills. The two solutions are due to slightly different input conditions which result in relatively larger differences in the energetics of the flow: the flow with higher kinetic energy goes over the top of the hill, whereas the flow with lower kinetic energy goes around. In the traditional dispersion modeling approach only one plume trajectory would have been calculated. As our illustration shows, there is a 50% chance that the calculated trajectory is the right trajectory, but a 50% chance that the plume follows a very different trajectory. Use of the ensemble forecast method will allow emergency response planners to better understand the possibilities of where the plume might go and hence could ultimately save lives.

Urban Canopy Parameterizations

Buildings and urban land use significantly affect plume transport and dispersion, altering the wind, temperature, turbulence, and radiation budget fields. The transport of CB agents over large distances must be handled by regional atmospheric codes; these codes, however, cannot "see" the buildings explicitly because their spatial resolution, or grid size, is on the order of kilometers. We have developed urban canopy parameterizations that allow the atmospheric models to "feel" the effect of the buildings without explicitly resolving them. We have accomplished this by adding drag to the momentum equations, mechanical turbulence production to the turbulent kinetic energy and turbulent length scale equations, anthropogenic and roof heat to the temperature equation, attenuation and trapping due to buildings to the short- and long-wave radiation equations, and urban land use properties to the surface energy budget equation. We have recently incorporated these urban canopy parameterizations into the COAMPS mesoscale meteorological code, which is the prognostic atmospheric code in the emergency response atmospheric dispersion modeling system under development. As shown in the figure on the previous page, the results indicate that the urban
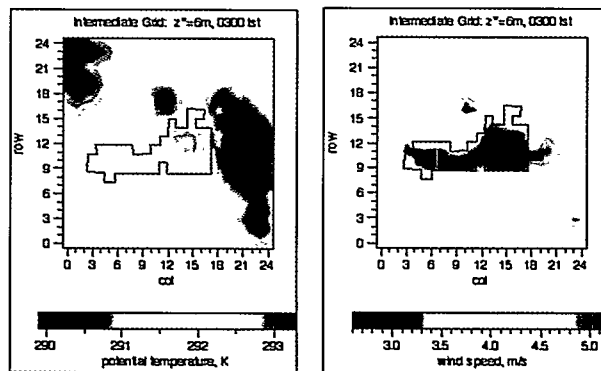
Heating (left) and wind speed (right) reduction associated with the urban area are simulated with an atmospheric code using an urban canopy parameterization. The Dallas-Ft. Worth area is denoted by the black outline. The reduced winds and enhanced mixing over the city will significantly alter the plume transport and dispersion of a CB agent.

canopy parameterization is able to simulate the night-time urban heat island phenomenon and the slowing of the wind over the urban area. For CB releases within cities, accounting for the urban influence on the meteorological fields is essential for accurately quantifying the transport and dispersion of the CB clouds. The modeling system now has this capability.
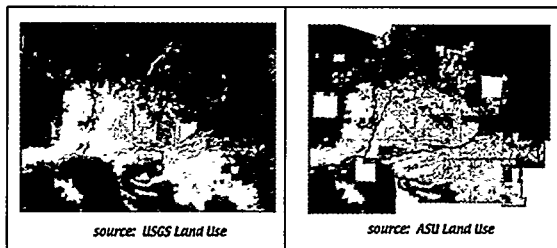
Future Outlook

Dispersion Forecast Uncertainty

Ensemble forecasting for dispersion applications is a relatively new area of research. We are building on the methods developed at the National Center for Environmental Prediction to generate the meteorological ensembles, and we are extending our methods to create an enhanced ensemble capability. We are exploring pattern recognition techniques and other analysis methods for displaying the results and determining the uncertainty. We expect to demonstrate our ensemble capabilities in exercises planned for later this year.

Urban Canopy Parameterizations

Research needs to be performed in three overlapping areas: model physics improvement, urban database generation, and model/parameterization validation. Our approach is to keep the parameterizations as simple as possible while adequately describing the urban canopy phenomena. We need to be careful not to add too much detail into the parameterizations, as this means more input data are required at higher fidelity and such data currently are just not available. As shown in the figure to the right, for example, we are finding that traditional urban land use data sets often contain outdated data. LANL and LLNL scientists will continue to work closely together on urban canopy parameterization development, validation, and application.

A quantitative understanding of the uncertainty in the computed plume trajectories of CB agents will be extremely valuable for emergency response decision makers.

Accounting for the influence of buildings on the transport and dispersion of the toxic plume will be essential for accurate assessment of CB terrorist attacks in urban environments.

source: USGS Land Use   source: ASU Land Use

Urban land use map for Phoenix shows significant differences between (top) readily available U.S. Geological Survey (USGS) data and (bottom) hard-to-obtain datasets like this one collected by Arizona State University. For this example, using the USGS data would significantly underpredict the impact of the urban area on the transport and dispersion of the CB plume.

Modeling and Prediction

Publications

M. Brown, "Urban Parameterizations for Mesoscale Models, to be published in *Mesoscale Atmospheric Dispersion*, Z. Boybeyi, Ed. (WIT Press, 1999), LA-UR-99-5329.

M. Brown and M. Williams, "An Urban Canopy Parameterization for Mesoscale Meteorological Models," Amer. Met. Soc., 2nd Urban Env. Conf., Albuquerque, NM (1998).

National Atmospheric Release Advisory Center Internet Remote Access System

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Objective

The objective of this work is to develop software which will allow remote access to National Atmospheric Release Advisory Center (NARAC) modeling capabilities at Lawrence Livermore National Laboratory (LLNL) with standard desktop and laptop computers using Web/Internet technology. Such a capability would provide emergency managers and first responders not only with easy access to the advanced atmospheric modeling tools developed by CBNP and integrated into the NARAC but would also provide them with expert assistance and advice from the NARAC operations center. The NARAC capabilities include (1) real-time global meteorological observations and forecast data collection, (2) U.S. and global geographical databases (e.g., maps, terrain elevation, population data), (3) 3D complex-terrain multiscale meteorological data assimilation, meteorological forecast and hazardous-material dispersion predictions, and (4) expert technical and scientific staff with training and experience in operational modeling and assessment of hazardous material releases into the atmosphere. Potential users of this system include emergency planners, first responders, emergency managers, operational staff, and research scientists. When this work is completed, users of commonly available desktop and laptop computers will be able to download software from NARAC that allows them to (1) enter the information necessary to describe a real or potential atmospheric release of chemical or biological material, (2) electronically send (over the Internet or any other communications link using Internet protocols) this information to the central NARAC system to perform model calculations, and (3) quickly receive maps indicating the areas and population predicted to experience health effects.

Recent Progress

A prototype version of the NARAC Web/Internet remote access system was completed and demonstrated in FY99. This software application suite provides the ability to enter basic information about an atmospheric release of chemical or biological material, communicate with the NARAC operations center, and view maps of NARAC-predicted health hazard zones (see figure to the right) The graphical user interfaces provide a look and feel that will be familiar to most personal computer users, greatly reducing the initial learning curve.
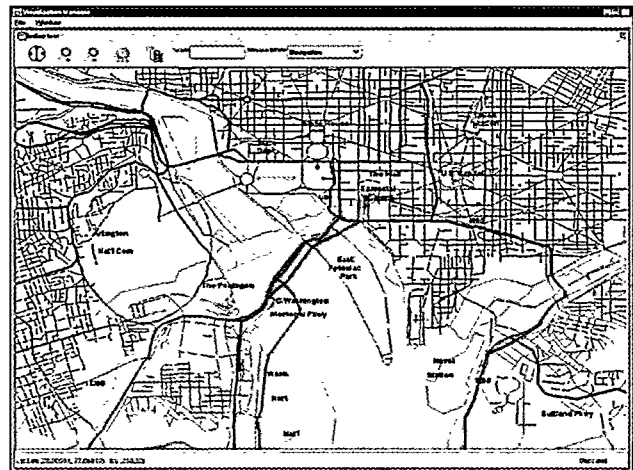
Example of a display from the NARAC Web/Internet remote access system software application of health hazard zones.

In order to make advanced atmospheric modeling and prediction tools readily available to first responders and emergency managers, remote access to the National Atmospheric Release Advisory Center (NARAC) is being developed using Web/Internet technology.

The software allows viewing, editing, archiving, and submission of basic information about the location, time and type of chemical or biological material released. The user may point to the location on an interactive map. The basic graphical display tool allows viewing and archiving of model predictions along with maps that are preloaded on the remote user's computer. This tool allows a user to archive, retrieve, view and interact with maps and model predictions (i.e., pan, zoom, change layer drawing order, select an area). A communication monitor provides status on the progress of the NARAC central system model calculation and product transmission. The ability to complete a "round-trip", from event scenario input to model product delivery, has been successfully demonstrated.

A key element of the remote access system is Java, a platform independent programming language. Software written in Java runs on any Java-equipped platform without modification (the platform must support a current version of the Java Virtual Machine, which is free for most platforms, including Windows 95/98/NT, OS/2, MacOS and most UNIX systems). As a result, most users will have at least one platform that can be used as a client machine (e.g., a personal computer running the Windows 98 operating system and user-provided Internet access).

We have investigated and tested commercial software installation technology (InstallAnywhere by ZeroG) to aid in web-based installation of remote client software. This technology proved to be a very promising mechanism by which a user can download and install the NARAC remote access software over the Internet using a web browser.

Future Outlook

The future development of the NARAC remote access system involves expanding the capabilities, security, performance and reliability of the prototype system completed in FY99. This work will include expanding the system's ability to describe potential CB releases, developing a geographic data distribution system, completing and enhancing the graphical output, developing direct communications with the new-generation NARAC central system, adding necessary computer security, expanding support for simultaneous users, integrating a rapid local modeling tool, developing interfaces to local sources of meteorological and CB sensor data, developing a web site (e.g., for software and user guide distribution), and developing a model product distribution mechanism.

Aqueous Foam for Mitigation and Decontamination

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Objectives

A nontoxic, noncorrosive aqueous foam with enhanced physical stability for the rapid mitigation and decontamination of CB agents has been developed at Sandia National Laboratories. This technology is attractive for civilian and military applications for several reasons including:
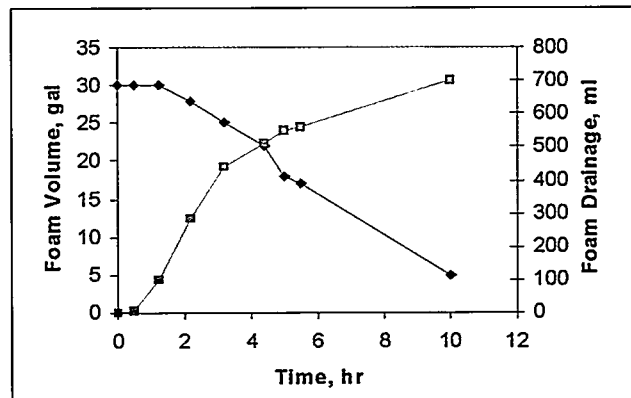
- It requires minimal logistics support,

- A single decon solution can be used for both CW and BW agents,

- Mitigation of agents can be accomplished in bulk, aerosol, and vapor phases,

- It can be deployed rapidly,

- It exhibits minimal health and collateral damage,

- It is relatively inexpensive, and

- It has minimal run-off of fluids and no lasting environmental impact

Our foam can be delivered by various methods. One preferred method is based on an aspiration Venturi effect, which eliminates the need to pump additional air into a closed environment and minimizes the transport of CB agents to uncontaminated areas.

As shown in the figure below, high expansion ratios and low rates of drainage have been achieved with our aqueous foam formulation.

Deployment of the Sandia chem-bio decontamination foam from fire-fighting equipment.


Foam stability vs. time.

Recent Progress

Results to date has shown effective decontamination of both CW and BW agent simulants and live agents on contaminated surfaces and in solution. For example, the figure below shows rapid decon of GD, VX, and HD on paper. Nuclear magnetic resonance (NMR) results indicate P–S rather than P–O cleavage in the VX simulant as desired to prevent the formation of toxic byproducts. Other experimental work has demonstrated that our foam effectively decontaminates CW agent simulants on surfaces such as wood, glass, carpet, concrete, asphalt, and CARC (chemical-agent-resistant coating). Testing has also shown that our foam neutralizes thickened agent simulants as well.

The foam has been shown to effectively decontaminate both CW and BW agent simulants and live agents without the production of toxic by-products.
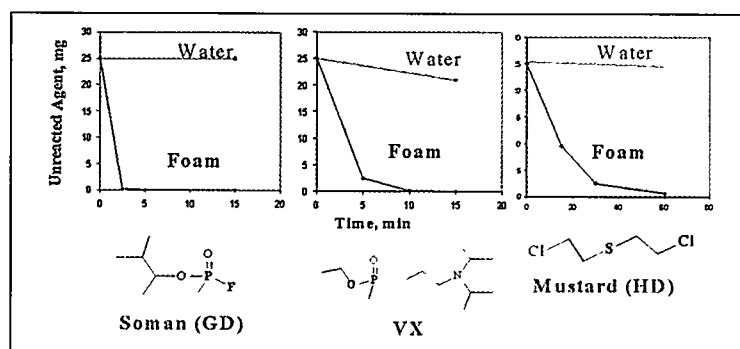
Decontamination of GD, VX, and HD on paper.

More recent results have shown that our foam effectively kills anthrax spores. The figure below shows 99.99999% kill of the spores after a one hour exposure to the foam solution. Additional testing has also demonstrated that our foam is also effective in killing vegetative cells of *Erwinia herbicola* and bacterial viruses (MS2) which are simulants for plague and smallpox.

Results to-date demonstrate rapid neutralization of sarin, soman, VX, and mustard as well as complete and rapid kill of anthrax spores.
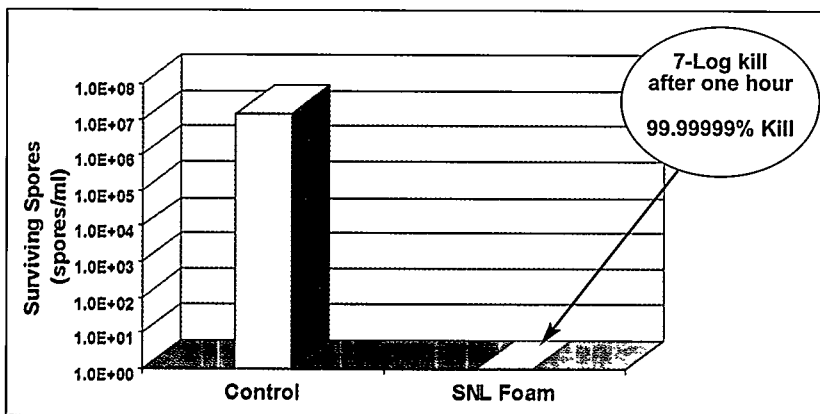
Anthrax spore kill.

One overall objective of the CBNP program has been to develop technologies for decontamination/restoration of three types of facilities:

open, semi-enclosed, and enclosed. Each of these types of facilities may require that our aqueous formulation be deployed by a different method (e.g., as a foam, spray, or fog) to achieve optimal performance and minimize collateral damage. At this time, deployment of the formulation as a foam is the most advanced. Our foam has been successfully deployed by small fire-extinguisher-type units (pressurized by CO_2 cartridges), by hand-held units which are pressurized by a connection to a fire hydrant, and by large military-style pumps. However, we are also developing methods to deploy the formulation as a spray and as a fog both of which may be more applicable for semi-enclosed and enclosed facilities. To date, our formulation has been successfully deployed through spraying and fogging devices in preliminary experiments.

Future Outlook

In FY00, the program will focus on foam optimization, live agent testing on various surfaces, further engineering of foam deployment systems, field testing against BW simulants, alternative deployment methods (misting, fogging, etc.), and commercialization efforts.

Field Tests

We have submitted our foam to the Edgewood Chemical Biological Center (ECBC) for a Department of Defense sponsored study of our technology. ECBC is located at the U.S. Army Aberdeen Proving Ground in Maryland. Part of the study included a small-scale reaction rate test wherein the liquid used to produce our foam attained complete destruction of chemical agents GD, VX, and HD within one hour and in many cases, within 10 minutes.

Publications

M.D. Tucker, "Aqueous Foam for CB Decontamination" *Proceedings of International Workshop on Decontamination in a Chemical or Biological Warfare Environment*, Durham, UK, September 7–9, 1999.

The nontoxic, noncorrosive formulation can be rapidly deployed through commercially available foam–generating equipment and could be used at the scene of a CB agent attack by both first responders and by decontamination teams focused on facility restoration.

Decontamination and Restoration

Universal Oxidation for Decontamination: L-Gel System Development and Deployment

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Objectives

The general philosophy of this work is to develop an integrated set of decontamination methods and tools that will work on the major CB threat agents. The work includes some near-term techniques that can be demonstrated within a year and implemented soon thereafter as well as longer-term research objectives. It is recognized that there is a balance between somewhat less effective methods which can be demonstrated quickly and more effective ones which may require a much longer time to fruition. The optimum goal of this study is to find a single decontamination system for chemical and biological agents which is nontoxic, noncorrosive and easily deployable.

One of the goals is to have decontamination systems that might be used by first responders as well as more complete systems to be used by specialized decontamination teams. Therefore, the overall project goal is to develop better decontamination methods that can be quickly implemented by these organizations. This includes early demonstrations and field work with companies or other government agencies that can identify implementation concerns and needs. The approach taken in this work is somewhat different than the standard military approach to decontamination. In a battlefield scenario, it is critical to decontaminate to a useful level in a very short time so the soldiers can continue their mission. In a domestic urban scenario, time is of less consequence collateral damage and recertification (public perception and stakeholder acceptance) are of much greater importance.

The specific objective of the LLNL work to date has been to evaluate various oxidizer systems as reagents to allow for detoxification and/or degradation to nontoxic environmentally acceptable components rather than necessitate complete destruction. Detoxification requires less reagent material than total oxidation, thereby reducing the logistical burden for a decontamination team. Since we also wanted to maximize the contact time between the decontaminating reagent and the contaminant agent, we selected gelled reagents as the primary carrier material. Gels have the additional advantage of adhering to vertical and even the underside of horizontal surfaces, such as ceilings and walls. Lawrence Livermore National Laboratory, over a period of twenty years from the late 1960's to the late 1980's, developed a series of extrudable high

Oxone gel successfully combines oxidation followed by hydrolysis for CB detoxification.

explosives based on the gelling of polar energetic liquids. While never going into production, this development served as an experience base for formulation, characterization and dispersal system design and fabrication. It was a logical step, therefore, to adapt this work to the gelling of aqueous oxidizers for candidate BW/CW decontaminants.

Recent Progress

Various aqueous phase oxidizers were evaluated to include potassium permanganate, peroxydisulfate, peroxymonosulfate, hydrogen peroxide as well as both acidic and basic hypochlorite. These types of materials allow for various dispersal and/or application methods, depending on the particular scenario (e.g., outdoors, semi-enclosed, or indoor). These oxidizer systems can be deployed in various carriers, such as compatible liquid and water-based spray systems, or incorporated into compatible gels to meet deployment needs.

Experimental testing at LLNL has shown that RO• (peroxyl) oxidizers are the most effective for complete CW and BW decontamination. The primary decontamination system now under development at LLNL is based on the commercial oxidizer "oxone" manufactred by DuPont. The active ingredient is potassium peroxymonosulfate. Our work built on previous research at Edgewood Chemical and Biological Center (ECBC) that demonstrated the effectiveness of aqueous oxone in decomposing both VX and mustard type agents. While decomposition of "G" agents occurred, the reaction was very slow because of the low pH of the system. The acidic pH is necessary, however, in the case of VX, where it leads to the protonation of the amino nitrogen and enhances the oxidation of the sulfur. Once the sulfur is oxidized the P–S bond is easily and irreversibly hydrolyzed, detoxifying the "V" agent. A similar oxidation of the sulfur in mustard facilitates the hydrolysis of the C–Cl bonds.

LLNL has incorporated an amorphous fumed silica gelling agent into the oxone solution. Experimental testing on both surrogates and real chemical agents has further shown that only RO• (peroxyl) oxidizers are effective for complete CW decontamination. This formulation was also found to be effective for all biological surrogates/spores as well as live vaccine strains (B. anthracis Sterne). These types of gels have the advantage of being compatible with strong oxidizing agents as well as maximizing contact time due to their thixotropic nature. The viscosity of the system can be varied depending on the application. Under shear forces, they have very low viscosity; when there is no shear, they become very viscous. This allows the gel to be sprayed using an atomizing nozzle and when the gel is dry (1–6 hrs) it can simply be vacuumed up and discarded. The final formulation is relatively noncorrosive (pH approximately equal to that of vinegar) and EPA testing on the residual materials from surrogate experiments shows the residues to be nonhazardous.

We have demonstrated the effectiveness of aqueous oxone in decomposing both VX and mustard type agents.

The L-Gel system has been tested on a variety of different materials as would be expected in an actual decontamination scenario. To date, the gelled system has been tested with a complete suite of CW (Amiton, DPCP, CEES) and BW (*Bacillus globigii, Erwinia herbicula*) surrogates on substrates of fiberglass, varnished wood, painted steel (acrylic paint), and indoor/outdoor carpet. The surrogate agent is placed on the test
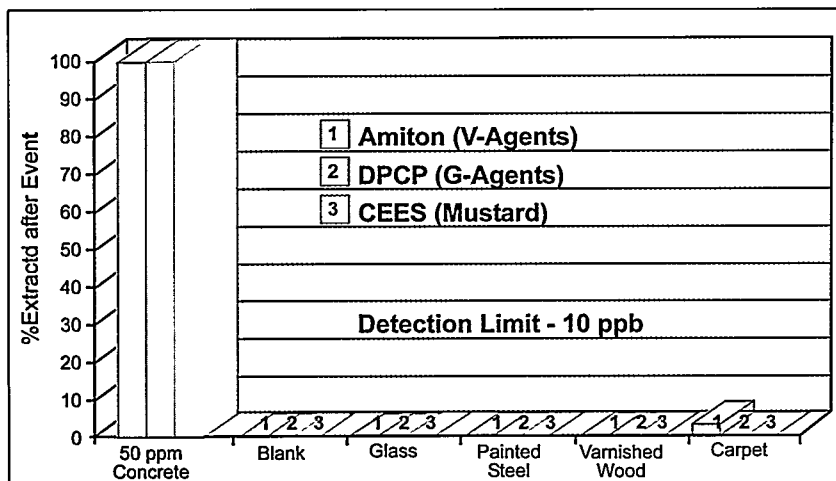

Gel with "oxone" inhibits spore growth.

Gel without "oxone" exhibits spore growth.
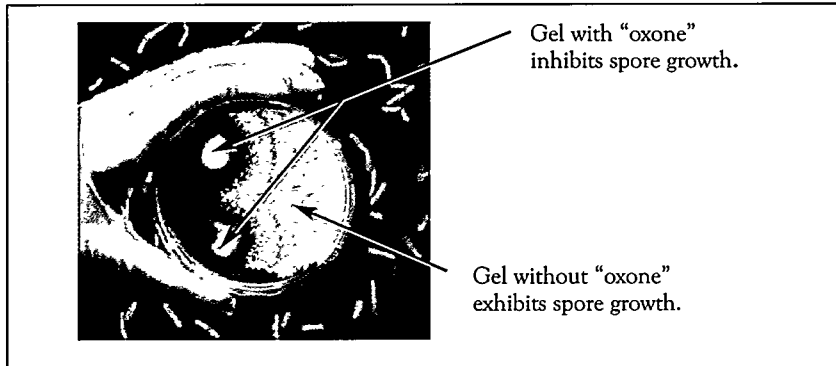
**Biocidal effect of L-Gel on *B. Globigii* spores.**

material and the reagent gel is added to the surface and allowed to dry. In every case but one, no surrogate material was detected after treatment. (A small amount, approximately 5% of the Amiton was detected after extraction from the carpet material.) Similarly, no BW surrogates were detected after treatment. All experiments were done with appropriate laboratory controls and standards.

The U.S. Edgewood Chemical and Biological Center (ECBC) has been involved in the laboratory evaluation and field testing of L-Gel. Real CW agent laboratory testing has been completed by the ECBC on the above-described surrogate materials. The L-Gel reduced VX, HD and GD to below detectable limits on all surfaces tested, with residuals of

L-Gel reduced VX, HD and GD to below detectable limits on all surfaces tested, with residuals of GD found on the painted surfaces.

1 Amiton (V-Agents)
2 DPCP (G-Agents)
3 CEES (Mustard)

Detection Limit - 10 ppb

%Extractd after Event

100
90
80
70
60
50
40
30
20
10
0

50 ppm Concrete   Blank   Glass   Painted Steel   Varnished Wood   Carpet

L-Gel effectively decontaminates chemical agent surrogates for VX, sarin and mustard on various surfaces.

B. globigii spores

(Detection limit < 100 cfu / ml)

Controls: 5 x 10^8 cfu / ml
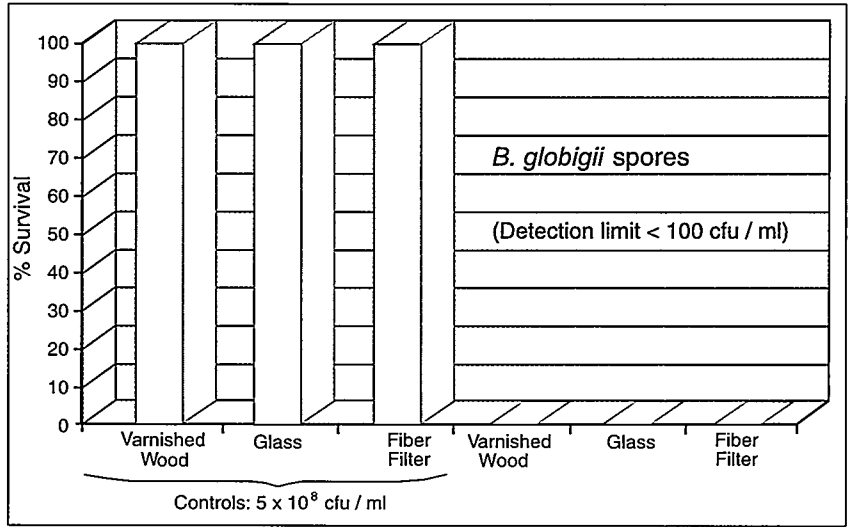
L-Gel effectively decontaminates *B. Globigii* spores to below detection limits on various surfaces.

GD found on the painted surfaces. Agent concentration used for experimental testing was 25 to 50 gms per square meter. Required gel material for complete decontamination is about 200 gms per square meter at a thickness of 5 mils. The most significant finding and advantage of the L-Gel material is surface catalysis effected by the gelling agent on G agent hydrolysis. The rate of hydrolysis of G agents by the gelled aqueous oxone, at pH 2, is nearly as great as normally observed in aqueous base. This property allows for the decontamination of G agents, V agents and mustard with a single reagent.

L-Gel has an effective shelf life of 6 to 8 months, allowing it to be pre-mixed. L-Gel is thixotropic (i.e., it liquefies upon vibration and solidifies when left standing). Therefore, after liquefaction by manual or mechanical shaking, it can be applied using any commercially available paint sprayer. L-Gel clings to walls and ceilings and does not harm car-
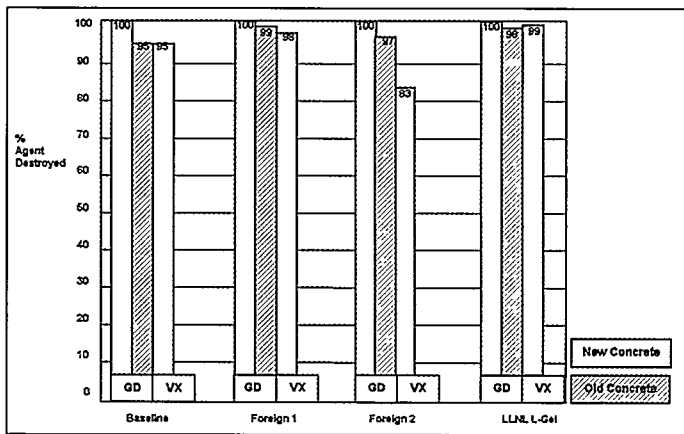
L-Gel system was more effective against VX and GD than the baseline U.S. military method (HTH) in field trials.

pets or painted surfaces. L-Gel has been independently tested against all classes of chemical agents and has been found to be as effective as the best military decontaminants. L-Gel has also been shown to be as effective against biological materials, including spores, as the best available commercial disinfectants.

Future Outlook

During this next year we will finalize all laboratory and field testing on live CW and BW agents. Laboratory testing is under way on live vaccine-strain biological agents to verify our surrogate findings. Preliminary tests show that the L-Gel is effective against the toxin surrogate ovalbumin, and real toxin decontamination also needs to be verified in a follow-on field test.

Efforts are also under way to begin technology transfer activities for the L-Gel formulation to a commercial and/or military partner. Work continues to address potential new gas and/or aerosol phase systems to meet other technical requirements.

Tests show that L-Gel is effective against the surrogate *B. globigii*.

Field Tests

DOD Commodity Area Field Tests, January 1999
Field experiments on VX and GD validated laboratory findings for those types of agents on both concrete and asphalt surfaces. Even though contact time was limited to 30 minutes, the L-Gel system was more effective against VX and GD than standard military methods (i.e., HTH). The L-Gel was successfully applied using an electric paint sprayer.

Dugway Proving Ground BW Field Test, December 1999
The test objectives were to:

- Develop optimal techniques for biological warfare agent simulant contamination of six types of material surfaces.

- Develop techniques for sampling/recovery of BWA simulant contamination of materials surfaces.

- Compare the ability of several decontamination materials to inactivate a BWA simulant. LLNL tested the L-Gel formulation and results are still pending.

Publications

Raymond R. McGuire, et al., "Oxone Based Decontamination," *Proceedings of International Workshop on Decontamination in a Chemical or Biological Warfare Environment*, Durham, UK, September 7–9, 1999.

Raymond R. McGuire, et al., "Oxidative Decontamination" *NATO/ISTC Advanced Research Workshop on Environmental Aspects of Converting CW Facilities to Peaceful Purposes and Derivative Technologies in Modeling, Medicine and Monitoring* (KLUWER, 1999), in press.

Raymond R. McGuire, et al., "The Results of Agent Testing of a Decontaminating Gel", *Proceedings of the 1999 Joint Service Chemical and Biological Decontamination Conference*, Nashville, TN, June 8–10, 1999, in press.

Decontamination Using Fenton-Related Reagents and Reactive Gases

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Objectives

Following the release of chemical or biological warfare agents, a decontamination process must be executed. Releases in certain facilities may warrant the use of a very aggressive decontamination strategy followed by dismantlement and disposal of the facility. However, one must also prepare for the decontamination of high-value facilities. We are developing decontamination techniques applicable to semi-enclosed and controlled spaces, including high-value facilities. Clearly, the optimum is a single decontamination method that is effective against all threats under all environmental conditions and that is nontoxic and noncorrosive. However, this simply may not be possible, and it may prove necessary to have a suite of decontamination systems. In developing decontamination techniques for domestic applications, we recognize that the rules of engagement in civilian facilities are somewhat different than the traditional military approach. In the battlefield scenario, it is critical to decontaminate to an acceptable level in a very short time so that the soldiers can continue the mission. In the domestic urban scenario, time is probably of less consequence but collateral damage, public perception, and recertification are of much greater consequence. Thus, social, political, and regulatory issues must be considered. For example, decontamination reagents cannot be environmentally hazardous materials, such as carcinogens, unless they have a short lifetime and decompose without endangering civilians or preventing recertification. In the domestic case, decontamination procedures must be defensible to regulatory agencies and to a public which may not be fully informed. Our work has focused on powder-based biocides that can be activated simply by mixing with water. We are also examining reactive gases as a means of decontaminating hard-to-reach places. Gases could also be used in conjunction with other decontamination techniques to complete a decontamination exercise.
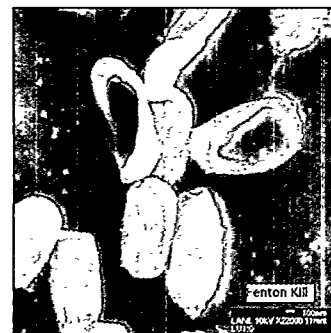
Recent Progress

We have examined modified Fenton reagents for the decontamination of bulk quantities of agent and for contaminated surfaces. Fenton (and related) reagents are potent biocides based on chemically produced free radicals (conventional Fenton chemistry involves oxidation of a transition metal ion, such as iron or copper, to dissociate hydrogen peroxide into hydroxyl radicals). More specifically, we have found that a simple combination of a copper salt, vitamin C, and table salt, when dissolved in water, form a very potent biocide. Evidence suggests that the dissolved copper facilitates free-radical cleavage of DNA and proteins (toxins). This rapidly renders the bioagents nonlethal. We have successfully used this reagent directly as an aqueous solution and have also made persistent and effective foams with it by employing fluorinated surfactants. This reagent also lends itself to long-term storage and easy activation—just mix the solid powders with water and

Reactive gases provide a practical means for decontaminating hard-to-reach areas such as cracks, crevices, and air ducts.

disperse. We have also begun exploring applications of this reagent to decontamination of personnel and medical devices. Bacterial spores killed by this reagent are shown in the figure below.

While liquids, gels, and foam-based reagents should be effective in decontaminating exposed surfaces, we believe that reactive gases will be necessary to complete a full decontamination since gases are the only practical means of getting into small cracks, cul-de-sacs, microporous materials, and air ducts. Using one gas (ozone) as a baseline, we have identified a number of practical issues that might affect the use of gases. These include the effects of relative humidity and agent purity. We have also assessed the collateral damage induced by the gas (an important consideration in high-value facilities). We have found that gaseous ozone, under the right environmental conditions, can be

With the modified Fenton reagent, spores are generally deactivated via internal mechanisms.

> Carefully selected gases, when used under the proper conditions, show promise of decontaminating a variety of agents with an acceptable amount of damage to infrastructure and sensitive equipment.

an effective reagent against biological agent surrogates in as little as one-half hour. Bacterial spores appear to be slowly dissolved upon contact with ozone, as shown in the electron micrograph below. However, due to the difference in density between gases and liquids, the rate of


Spores exposed to ozone gas appear to be slowly solubilized or melted.

destruction of chemical agent surrogates is considerably slower. Times on the order of a day or two may be required for the most persistent chemical agents. For releases within civilian facilities, we believe these times are still acceptable. We have also examined the effects of this gas on optical and magnetic storage materials and on selected electronic equipment (computers). In most cases, there appears to be window within which one can deactivate the agents yet still preserve key data and equipment.

Future Outlook

The modified Fenton work is now being pursued via an industrial collaboration. For reactive gases, we will initiate a study of other candidate gases (for example, chlorine dioxide and vaporous hydrogen peroxide) and continue collateral damage studies on an expanded set of materials. We will also pursue gas-phase kinetics measurements, which would apply to the treatment of contaminated air within a facility. Similarly, we hope to determine the feasibility of using these gases in

small decontamination chambers to decontaminate sensitive equipment and suspicious packages. We also intend to elucidate the chemical mechanisms governing the reaction between the gases and chemical agents (both surrogates and, ultimately, live agents). This work will enable us to identify the chemical by-products and their residual toxicity. Finally, we intend to correlate our results into a form suitable to responders and planners. For example, a computerized expert system could be developed and distributed. This software tool would point the planner towards the best strategy for a given scenario.
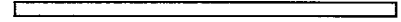
Atmospheric Pressure Plasma Technology for Decontamination

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Objective

The objective of Atmospheric Pressure Plasma (APP) technology for CB decontamination is to detoxify a surface film of CB agent using the reactive stream generated by a plasma composed of innocuous gases, such as helium and oxygen. The advantages of this approach are:

- Harmless storage gases can be used for the detoxification process.
- The method is dry and does not affect most sensitive equipment.
- No residual cleanup is required.
- The process is fast and can be applied to localized areas as needed.

The reactive species, typically atomic oxygen and metastable molecular oxygen, are generated by passing the feed gas through a plasma (e.g., an ionized gas consisting of ions, electrons and neutral particles) where it becomes chemically activated through collisions with energetic electrons. Because the reactive species generated by the plasma are short-lived, if they do not react with their target, they quickly deactivate back to innocuous gases and thus pose little or no operator safety or environmental concern.

APP decontamination fills a vital niche among the various decontamination methods because, unlike traditional methods, APP is dry and nondestructive to sensitive equipment, such as electronics, and irreplaceable objects, such as national treasures. APP would provide a fast and portable means of restoration of contaminated items for which the only current option is ultimate disposal. These devices would rely heavily on the novel Atmospheric Pressure Plasma Jet (APPJ) technology which has been developed at Los Alamos National Laboratory (LANL) over the past five years and was a winner of a 1999 R&D 100 Award.

Recent Progress

The APPJ is a unique nonthermal glow-discharge plasma operating at atmospheric pressure. The discharge uses a high-flow feed gas consisting primarily of an inert carrier gas, such as helium, and a small amount of an additive to be activated, such as oxygen. The feed gas flows between an outer, grounded cylindrical electrode and an inner, coaxial electrode powered at 13.56 MHz radio frequency. The electric field produced between the electrodes causes the gas to break down into a plasma state. While passing through the plasma, the feed gas becomes excited, dissociated, or ionized by energetic electron impact. Once the gas exits the discharge volume, ions and electrons are rapidly lost by recombination leaving metastable species (e.g., O_2^*, He^*) and radicals (e.g., O). These reactive species are then directed onto a contaminated surface at high velocity, where they can selectively

Atmospheric Pressure Plasma technology provides dry, fast, nondestructive, safe, portable, "green" decontamination.

"Cold flame" burns CB agents at low temperatures.

Development is focused on decontamination of sensitive equipment and materials which are readily damaged by other methods.

neutralize CB agents without damaging the underlying surface. Although the effluent of the APPJ, seen in the photograph below, may look somewhat like the flame of a Bunsen burner, its temperature can be maintained cooler than that of a hair dryer's exhaust. The reactive species of oxygen essentially "burn" many organic materials, such as CB agents, at these relatively low temperatures without damaging the underlying material.
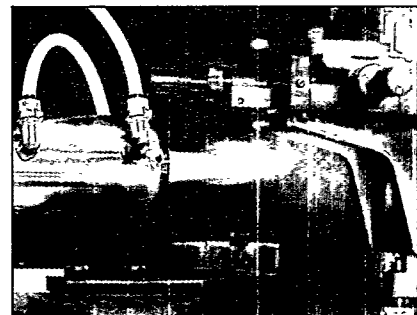
The LANL APPJ system.

The reactive effluent of the APPJ has been shown to kill *Bacillus globigii* (Bg) spores, a surrogate for anthrax, with a D value (time to reduce viability by a factor of 10) of 4.5 sec at an exposure temperature of 175°C and a stand-off distance of 0.5 cm. This is 10 times faster than hot gas at the same temperature and requires approximately 80% less energy input to achieve the same level of kill. Through active cooling of electrodes, we have also achieved a D value of 15 sec at an exposure temperature of just 70°C. At this temperature, there is essentially no pure thermal kill of bacterial spores and no thermal degradation of most materials.

The APPJ has also been shown to neutralize surrogate and actual CW agents. In the case of an HD simulant (2-chloroethyl phenyl sulfide, or phenyl half mustard), the sulfur was readily oxidized and the molecule was often dechlorinated, leading to a benign vinyl compound. Cleavage of the phosphorous–sulfur bond, a critical step in detoxification, has also been demonstrated in actual VX through a collaborative effort with the Edgewood Chemical and Biological Center.

Research efforts are now being directed toward reducing helium consumption and increasing the allowable stand-off distance. Alternative feed gas compositions have shown great promise in these efforts.

Future Outlook

The implementation process and knowing the best way to utilize a technology are crucial to the success of a technology. Implementation strategies directed towards sensitive equipment and materials are now being considered. One that is currently being developed is an APP decontamination chamber for decontaminating items that can be placed inside a closed system. By closing the system and recirculating the feed gas we can avoid high helium consumption and reaerosolization of agents. A closed system also makes it possible to use methods such as pressure reduction and/or pulsing to greatly increase penetration of reactive

species into contaminated equipment. Reduced pressure increases the lifetime of reactive species, whereas pressure pulsing helps the reactive species penetrate into crevices and cavities. The combination of heat, vacuum, forced convection, and reactivity should enhance the removal of agent off of surfaces, and what is not adequately neutralized within the chamber will certainly be destroyed as it passes through the discharge during recirculation. Our goal is to demonstrate acceptable decontamination on the inside as well as the outside of sensitive items such as computer systems, telephone switching equipment, power station equipment, and national treasures at reasonable temperatures (e.g., <100°C) in a reasonable period of time (e.g., approximately 10 min). We believe these goals are achievable with the current state of the technology, and continuing activity on fundamental APP studies (e.g., alternative feed gases) will yield additional process improvements.

Several techniques are also being evaluated for use in an APPJ for decontamination of sensitive equipment and materials, that cannot be placed inside a chamber. This device would be most useful for spot decontamination of interior spaces containing these items, such as airplanes, control centers for commercial communications, power and transportation facilities, and conventional offices. A portable system decontaminating the cockpit of a jet is depicted in the figure below.

APP is a dual-use technology with applications in sterilization, semiconductors, polymers, coatings and industrial cleaning.
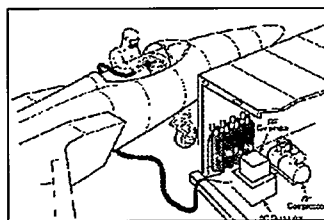
We have also been working in collaboration with the military to advance APP decontamination technology. The APP decontamination chamber is currently included in the military's Joint Services Sensitive Equipment Decontamination program, which will select promising technologies to be transitioned into the engineering phase at the end of FY00. We are also in the process of opening licensing of the technology to the private sector to establish an industrial base. APP technology has great potential in many areas including sterilization for the health-care and food industries, semiconductor plasma processing, modification of polymer properties, coatings for architectural glass and tool hardening, and as a replacement for hazardous solvents for industrial cleaning processes. The dual-use nature of this technology will accelerate its further development while providing ready suppliers for complete APP decontamination systems.

Portable APPJ system.

Field Tests

The APPJ was tested against VX at the Edgewood Chemical and Biological Center, in experiments held between September 1998 and May 1999.

Publications

H.W. Herrmann, G.S. Selwyn, et al., "Dry Decontamination of Chemical/Biological Warfare Agents using an Atmospheric Pressure Plasma Jet," *Physics of Plasmas* 6, 2284 (1999).

J. Park, I. Henins, H.W. Herrmann, G.S. Selwyn, J.Y. Jeong, R.F. Hicks, D. Shim, C.S. Chang, "An Atmospheric Pressure Plasma Source," *Applied Physics Letters* 76, 288–290 (2000).

How Clean is Clean Enough?

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Objective

The overall issue of "How Clean is Clean Enough?" and the methods by which this is determined (e.g., sampling and verification) are key to establishing effective and successful decontamination methods. Therefore, the primary goal of this effort is to establish a methodology to determine what level of clean up will be required to meet both regulatory and stakeholder needs. This is a very complicated issue that will impact technologies currently being developed for detection and decontamination. It involves assessing and evaluating the current available information and/or existing standards for CB decontamination and public reuse. This evaluation is necessary to begin laying the groundwork for establishing realistic clean up and verification criteria in the event of an actual terrorist incident.

In the context of a potential release of CB agents, three kinds of sites may bound the response and decontamination approach to be taken. They are:

- An outdoor site, such as a stadium or campus
- A semi-enclosed site, such as a subway or airplane hangar
- An indoor environment, such as an office building, home, or room

For outdoor sites, weather can be an important consideration in terms of an inability to contain contaminants, and dilution and natural attenuation may be among the most effective decontamination approaches. In contrast, indoor environments like buildings are likely to be more controlled systems, with ventilation systems that may also need decontamination, requiring the use of aerosols and/or gases. For indoor scenarios, public perception is a major consideration, and cleanup to "acceptable" levels may prove even more difficult since occupancy time can be significant. A semi-enclosed site is a hybrid of outdoor and indoor sites. Major considerations here are the positive effects of ventilation and the likelihood of short residence times for people. In particular, our overall objectives have been to:

- Benchmark and document current decontamination practices
- Identify and document technical and political/public issues
- Evaluate industrial hygiene models for toxicology applications
- Review current applicable regulatory guidelines to establish and/or develop appropriate clean up levels
- Determine necessary decontamination sampling and verification procedures
- Develop a framework for decision makers to determine whether actual or potential impact to human/animal health and/or property exists, and whether decontamination is actually needed

Achieving these objectives will ultimately help in establishing necessary cleanup levels, which drive the requirements for the decontamination technologies. In other words, if killing 90% of the biological cells satisfies the requirements of "clean", then a strong soap solution is sufficient for decontamination and the problem is solved. If, however, one requires a survival of less than one in 100 cells, the decontamination reagent must be extremely efficient. Thus, this effort is key to developing an implementation strategy to return facilities and/or infrastructure to the public sector for reuse.

Recent Progress

Potential agents were defined to include biological agents and toxins (as listed in the DHSS 42 CFR Part 7 Appendix A list of Select Agents) and chemical agents/ precursor compounds (as listed in Schedule I and II CWC). Literature searches and discussions with key agencies have shown that information is limited and, in some cases, contradictory in establishing safe criteria for the public exposure to CB agents. A summary of available dose values and exposure limits for specific CB agents has been compiled, primarily based on military data. Specifically, low-dose,

Scenario 1	Scenario 2	Scenario 3
Open (Stadium)	Semi-Enclosed (Subway)	Enclosed (Office, Home)
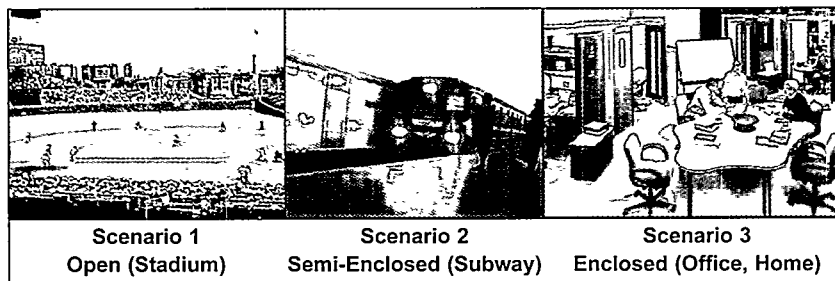
Decontamination issues will be scenario-specific.

long-term chronic toxicity data are not available for CW agents and/or most of their residual degradation products. Additionally, infectious dose levels for many of the biological agents of concern are not available and/or are controversial. Toxicological database tools were also found to be limited in their usefulness under these public sector conditions.

A number of factors must be considered in developing clean up standards for the general public, in particular for those individuals who may be at higher risk for illness and injury (developing fetuses, children, immunocompromised persons, etc.). In addition, much more strict criteria will be necessary for indoor, long-term reuse than for outdoor site scenarios where environmental factors will help to dilute and/or eliminate potential long-term health effects. Based upon our assessment to date, we recommend that a simplified risk assessment approach be utilized to gain a further understanding of necessary clean up levels based upon the sites as

described above. This would enable us to understand the relationship between the extent of the contamination and the applicable receptors and pathways. The distinction between sources, pathways, and receptors will be site- and/or scenario-dependent in many cases and therefore must be carefully defined to understand the real short- and long-term risks to human health/animal health, the environment, property or resources. Development of public standards or reentry criteria must consider public perceptions and appropriate risk communication techniques must be utilized to effectively implement recommendations based on scientific criteria.

We reviewed existing environmental regulatory limits which would be useful in establishing conservative cleanup levels for CW agents. Although very limited, it indicates "suggested no-action-required levels" in ranges of 50 to 700 ppb for similar residual byproducts. Simple modeling to determine soil cleanup levels has been done for the San Francisco, Washington DC, and Baltimore areas. Results indicate that a 20-/to 200-ppb level of soil clean up will be required for most of the nerve agents, depending on the actual depth to the water table.

For BW cleanup, the issues are more complicated since many organisms are indigenous and/or risk is a function of effective dispersion, exposure (i.e., inhalation), and infectivity. However, practices described by the American Industrial Hygiene Association in the "Biosafety: Reference Manual" and by the Centers for Disease Control in the "Biosafety in Microbiological and Biomedical Laboratories" may be adapted for use in the public sector.

In response to an interagency request, LLNL developed a conceptual Bio-Decontamination Decision Process to determine appropriate actions following a terrorist attack. The decision process flowchart represents a preliminary framework to address incident response functions/phases (e.g., notification, first responder response, restoration of operations and longer-term remediation). This allows a decision flow to address key issues and allows for decision makers to collect the necessary information to determine whether an actual or potential impact to human/animal health and/or property exists and whether further decontamination is needed. Furthermore, it addresses key decision points for regulatory and stakeholder review. The decision process has now been expanded to include chemical agents. A key step is to determine whether any decontamination is actually necessary or whether natural degradation/attenuation will in fact achieve low enough levels to meet public reentry criteria. Previous studies indicate for nerve agents on soil that values are essentially nondetectable after about three days, although the cited data represents only two soil types under high relative humidity. Of course, the initial concentration and other environmental effects must be considered when making this evaluation.

Available dose values and exposure limits for specific CB agents have been compiled.

Future Outlook

Ongoing tasking and future work is aimed at three specific areas as discussed below. These tasks should allow us to better integrate new decontamination technology research and development with end user needs.

We developed a conceptual bio-decontamination decision process to determine appropriate actions following a terrorist attack.
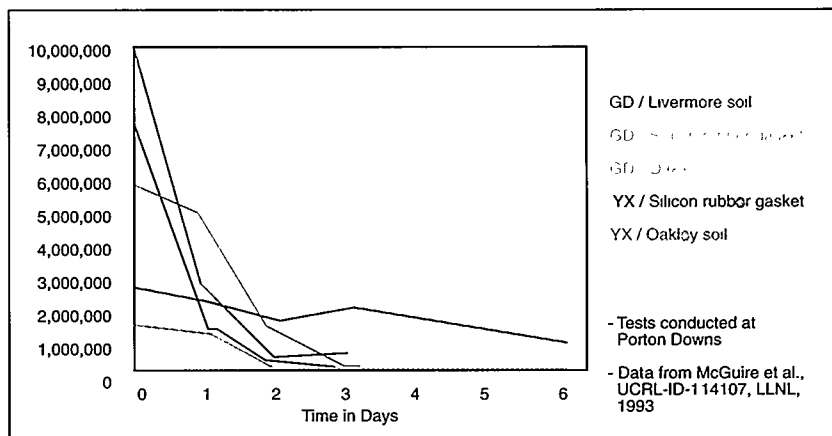
Natural degradation/attenuation of CW agents may be effective for outdoor cleanup scenarios.

Establish Necessary Cleanup Levels

During this next year, we will focus in on the necessary clean up levels based on completing some initial health risk assessments. To date our work has looked at requirements for air monitoring based upon existing regulatory public health guidelines as well as developing environmental regulatory limits for soil clean up. This more detailed approach will begin with a multimedia, multipathway dose assessment. For instance, the resuspension, subsequent multimedia transport, and fate of the substance or microorganism must be determined. This means that the ability of the contaminant to move onto and off of construction materials must be determined or assumed. The toxicology of the chemical or biological agent must also be evaluated, and human morbidity, mortality, and latency of effects need to be determined. Integrating multimedia transport and fate with multipathway exposure (e.g., inhalation, secondary ingestion, dermal absorption) and physiologically based pharmacokinetics (if available) for modeling toxicity should yield an estimate of noncarcinogenic hazard and carcinogenic risk, especially from short-term exposures.

Define Decontamination Technology Requirements

We will evaluate the ability of current decontamination technologies to remove and eliminate potential residual health effects due to a selected chemical or biological agent directly or indirectly placed on construction material as part of key scenarios involving contamination of an office building, subway, and stadium. This work should allow an identification of technology gaps and help in establishing overall future tech-

nological R&D requirements. LLNL will continue to work with the Interagency to refine and develop decision protocol tools for response to a CB event. We plan to work with the Interagency as appropriate and to participate in the planning and integration of upcoming ACTDs and/or DDAPs.

Define Sampling and Decontamination Verification Methods
FY00 work will address issues regarding field sampling and analysis and the development of a methodology for conducting these evaluations by a statistically based approach acceptable to the regulatory community. LLNL will apply its currently developed and approved methodology for decontamination of hazardous and radioactive contaminated facilities to the CB facility question. The actual techniques used to obtain a representative set of field samples will be different for chemical and biological agents. A very important aspect of this task involves having a documented sampling and verification procedure which is defensible should a real contamination event occur. This will be useful for many of the agencies concerned with terrorist or other chem/bio contamination incidents.

Publications

"Decontamination Issues for Chemical and Biological Warfare Agents: How Clean Is Clean Enough?" UCRL-JC-134030, LLNL (May 1999).

"Conceptual Decontamination Decision Process Following a CB Terrorist Attack," UCRL-AR-131181, Rev 1, LLNL (March 1999).

Ellen Raber, et al., "The Results of Agent Testing of a Decontaminating Gel", *Proceedings of the 1999 Joint Service Chemical and Biological Decontamination Conference*, Nashville, TN, June 8–10, 1999, in press.

Our work focuses on developing environmental regulatory limits for soil clean up.

We will evaluate the ability of current decontamination technologies to remove and eliminate potential residual health effects due to a selected chemical or biological agent.

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Objectives

The PROTECT program (Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism) is a systems approach to CB infrastructure protection. As such, PROTECT creates an integrated set of tools by combining the best existing and emerging technologies. The purpose is to save lives during an incident and restore operations as quickly as possible. PROTECT covers all time frames from pre-event planning and preparation, through crisis management and response, to forensic analysis and decontamination. PROTECT provides a wide array of tools for dealing with all aspects of the problem, including vulnerability assessment, training, detection and warning systems, crisis management, and passive and active countermeasures. Demonstrations are planned in at-risk facilities including subways, airports, high-threat buildings, and the interior components of special events. The top-level goals of the program are:

- Development and demonstration of technical systems
- Partnership with facility operators
- Integration of DOE and other technologies
- Coordination and dissemination of information and technologies to users and industry

Recent Progress

Progress to date has included steps forward in both the subway and airport demonstration programs. The PROTECT subway program is the most mature component of the infrastructure protection effort and is likely to be the site of the initial demonstration. The principal collaborator for this program is the Washington Metropolitan Area Transit Authority (WMATA).

Incident Response
Interim response guidelines have been developed with WMATA personnel, and they have been implemented in their standard operating procedures.

Sensing and Warning
A station has been chosen for a chemical agent test-bed. The test-bed will be used to evaluate the operation of detectors over long periods of time for false alarms and operational problems. Modeling has been done to understand the time of travel of agents from release points to various locations in

> PROTECT has joined with WMATA and MBTA to improve passenger safety on those subway systems in the event of a chemical agent crisis.

PROTECT is using computer models and "smoke" releases to site chemical agent detectors in a subway station. We are also developing computer control systems to monitor the detectors and to issue warnings should a chemical attack be detected.

After a chemical attack is detected, PROTECT is working with WMATA and MBTA to overcome problems of cleaning up and restoring service so that the subways are safe for public use again.

the station. This information has guided recommendations for chemical sensor architecture requirements. Smoke tests carried out in September 1999 have been used to better characterize airflow when trains enter and leave the station and to study airflow in a station when no trains have been present for a while. The data are useful for providing more precise guidance for detector placement. The figure below shows a scene from a smoke release in a train car reminiscent of the Tokyo subway attack in 1995.

An evaluation of chemical agent detectors has led to three commercial detectors being selected for testing/evaluation in a subway environment. Sensing and warning architectures, including detectors, gas-sampling manifolds, alarm buttons, and video systems, have been considered in the context of various types of release scenarios. To supplement these options, PROTECT is investigating the feasibility of an artificial intelligence system to analyze video and/or audio data to alarm after a chemical release.

Smoke released in subway traincar.

In addition to collaborating with subway systems to plan and execute chemical agent detector tests, PROTECT has been working with other government agencies supporting related studies. It is anticipated that the chemical detector test-bed will be operating by the spring of 2000.

Decontamination
Special site-specific requirements for subway decontamination after a chemical incident have been based on discussions with the Washington DC and Boston subway systems. Contacts with the National Response Team have led to discussions of subway needs versus National Response Team chemical agent decontamination capability and plans. This information is also being discussed with DOE decontamination researchers.

Transit Outreach
Communication with other subway organizations has continued. Substantial discussions have taken place with the Massachusetts Bay Transportation Authority (MBTA), operator of the Boston subway, on the CB problem and they have become active collaborators. PROTECT is developing a Memorandum of Understanding with the Federal Transit Administration of the U.S. Department of Transportation for collaboration on the subway CB problem.
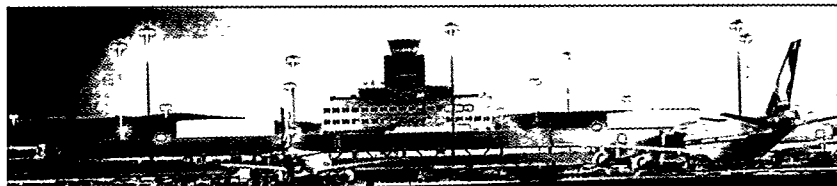
Airport Program

The PROTECT airport program was also initiated this year. Airport terminals are representative of an important class of large, conditioned interior facilities and are themselves vital infrastructure facilities. Initial work to guide the development of effective responses

One of many entrances to the Boston subway system.

to the CB threat to airports has begun in the PROTECT program. A collaborative agreement with an airport has been reached that focuses on characterizing the airflow in large terminal facilities. Initial data have been exchanged to permit planning for the characterization and analysis of the facility during its acceptance and prove-in testing by the airport. As with the subway interaction, support of broader emergency operations planning has also been initiated within the airport/PRO-TECT partnership.

Airport main terminal.

Future Outlook

A major activity will be the subway station detector test-bed study with possible inclusion of the prototype DOE μChemLab™ chemical detector. Development of alternative warning technologies, such as pattern recognition systems, will be pursued. A prototype of the responder tool Chemical and Biological Emergency Management Information System (CB-EMIS) is being developed to facilitate exchange of information among responders, the Incident Commander, and the Operations Control Center for a subway incident. In 2001, a single-station subway demonstration is expected to take place, possibly including a chemical agent response exercise.

The airport program is developing a comprehensive process as well as supporting technologies that will allow airport operators to develop effective operational response plans and will guide their decisions regarding investments in detection and mitigation architectures. The initial activities at the airport terminal employ testing and analysis to characterize the airflow within the large terminal facilities. This information will permit immediate planning for HVAC response measures that can minimize the impact of a chemical release. It will also provide

Building on the WMATA and MBTA work, PROTECT is working to include other subway systems and transportation facilities.

PROTECT is also active in protecting airports. Experiments are being planned in an air terminal to help develop strategies for enhancing public security in case of a chemical agent attack.

PROTECT will integrate sensors, computer models, control systems, and operational concepts to deliver a substantial increase in public security.

the basis for development of sensing and warning architectures. The test-bed results and analysis methodologies developed in conjunction with the subway projects are expected to be applicable to airport systems. The extensive video surveillance assets at the airport are expected to be an important component of the near-term CB sensing and warning system. The airport partnership will be building toward an integrated demonstration of linked detection and control systems.

Field Tests

Station Smoke Tests, September 1999
These tests were carried out with both qualitative (through video) and quantitative (through smoke detector measurements in real time) to measure and understand the movement of air and smoke through a station during a simulated release. Air speed and direction were measured at eight locations. The smoke has many of the same characteristics as a chemical agent in aerosol or vapor form. The tests are used, in part, to locate the chemical agent detectors in the test-bed as well as to assist in the development of improved fast-running station models for emergency response.

Publications

A.J. Policastro and S. Gordon, "The Use of Technology in Preparing Subway Systems for Chemical/Biological Terrorism," Commuter Rail/Rapid Transit Conference, American Public Transit Association, Toronto, Canada, May 22–27, 1999.

W.A. Swansiger and J.E. Brockmann, "Mitigation of Chemical Attacks in Enclosed Public Transportation Facilities," *Enforcement and Security Technologies Proceedings*, SPIE 3575, November 3–5, 1998.

BASIS: Defense Against Bioterrorism at Special Events

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Objectives

The Biological Aerosol Sentry and Information System (BASIS) is an effort initiated to provide medical and law-enforcement authorities with timely and accurate information about covert biological attacks. The system has been designed primarily for security operations at special events but will also support other operations, such as responses to previous intelligence or warnings.

The BASIS program provides significant enhancements to current monitoring capabilities using proven systems and technologies-including advances produced in DOE, as well as other agencies. The system is based on an "open architecture" to ensure that future technologies can be readily integrated. To ensure that the program can support existing and planned operational objectives, it is conducted in close cooperation with local, state, and federal health officials responsible for medical operations resulting from acts of bioterrorism.

Early detection and rapid responses can dramatically reduce the consequences of bioterrorism. Medical responses such as treatment, isolation, and identification of those who have been exposed will be most effective if initiated soon after the attack. Today, a covert release of biological aerosols would only be detected by means of standard public health surveillance procedures. These procedures depend on observation of unusual cases or conditions, and it could be many days before an attack is recognized and the agent is identified. By that time, it may be too late to save many of those who have been exposed if the agent is a fatal pathogen such as anthrax or plague. The major objective of the BASIS program is to greatly reduce the time needed for detection and identification of a covert biological attack. The top-level goals of the program are:

- Provide "detect to treat" timeline warning of biological agent attacks at special events through identification of agents and estimates of hazard zones
- Work closely with public health and other agencies that are customers for BASIS information
- Integrate DOE and other technologies into a robust system with very low false alarm rates
- Provide an integrated system that can be deployed in a timely manner at a wide range of special events

BASIS seeks to provide timely detection and warning of biological attacks.

BASIS was formerly known as the Sentry and Consequence Management Information System (SCMIS). The name of the program was changed to BASIS to better represent the current program definition.

Recent Progress

BASIS activities in FY99 focused on customer interactions, definition of the overall architecture prototype tool development and field exercise

support. This effort culminated in a comprehensive plan for demonstrating the BASIS system. After completion and a successful demonstration a decision will be made for deployment at a large special event.

Customer Interactions

The primary targeted customer for BASIS information is the public health community. Accordingly, we have worked closely with public health officials in the Public Health Service, the Centers for Disease Control, the State of Utah and Los Angeles County in understanding the information that would be of most use in deploying an effective medical response to a bioterrorism attack at a large special event. Through development of prototype decision support and training tools, described below, we have elicited information from public health officials that was incorporated directly into defining the functions and structure of the BASIS system.

Although we have primarily targeted public health officials, other agencies are interested in the information provided by BASIS, since this information is not available from any other system. These agencies include other local public safety organizations, such as law enforcement, fire and emergency medical services and Federal agencies, such as the FBI and Secret Service. Accordingly, we have maintained contacts at relevant points within these agencies and organizations to ensure that they (a) are aware of the BASIS program goals and mission and (b) have an opportunity to provide input to the structure and function of the BASIS system.

Architecture Definition

Based on the above extensive customer interactions and technologies available on the time scale of the BASIS program, an architecture has been developed. This architecture is shown in the figure and consist of four major elements:

- Distributed Sampling Units (DSUs) - DSUs monitor aerosols and collect aerosol samples for analysis

- Field Laboratory - The Field Laboratory provides high-throughput analysis of aerosol samples using very sensitive techniques which provide high -specificity for selected biological agents. On demand testing is provided for a wide range of biological agents. The aerosol sample archive is also maintained in the Field Laboratory to provide a comprehensive history of aerosol at monitoring sites for ex post facto analysis.

- Command Console (ComCon) - The ComCon provides a capability for real-time monitoring of aerosol information from the DSUs and analysis results as they become available in the Field Laboratory. The ComCon will also provide a warning function
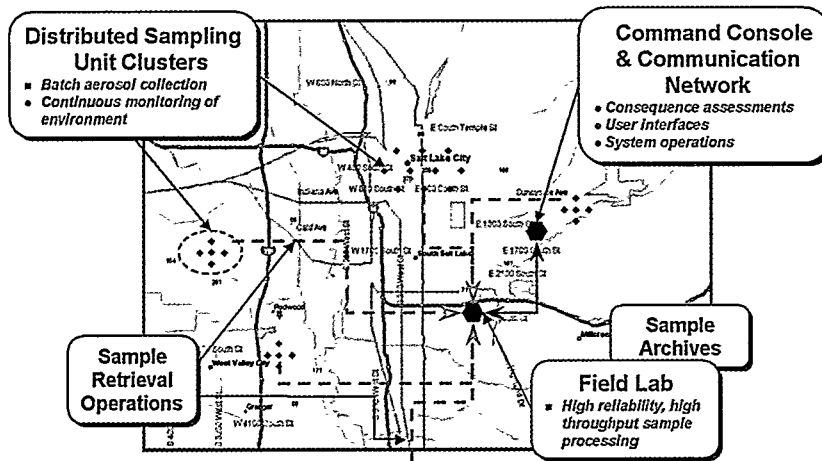
The BASIS Architecture.

into the Operations Center for the special event at which BASIS is deployed. Decision aids and planning and training tools are provided on the ComCon, including estimates of affected areas and populations, to provide public health and other officials with information to incorporate into their response to a biological attack.

- Communication System - The Communication System is structured to allow flexible and rapid deployment of BASIS to a special event. The communication system provides multiple and robust communication paths among the DSUs, the field laboratory and the ComCon.

The structure of the BASIS architecture was determined using input from the customers described above. The unanimous opinion that false positives are not acceptable. Thus, BASIS was structured to provide a centralized laboratory for highly specific and independent analyses of collected samples. The protocols for the field laboratory will be developed and extensively validated as a part of BASIS, prior to any operational field deployment. The customers also wanted to robustly monitor numerous locations. This led to the deployment of many inexpensive aerosol collection units and a centralized, high-throughput laboratory for analysis of the samples. The customers also wanted assistance in interpreting the results of the analysis and a capability for planning and training; this was incorporated in the ComCon. Finally, the BASIS system needs to be flexible enough to deploy at a succession of special events. Thus, the communication system is designed to be robust and flexible to accommodate a variety of deployment scenarios, including relocation of DSUs and other components during the deployment at a special event.

Prototype Tool Development

As a part of preliminary BASIS definition activities, prototypes of deci-

sion support tools were developed. These prototypes were used to elicit customer needs for the BASIS system. These tools included the Virtual Planner which allows customers to easily simulate the effects of release of a chemical or biological agent and sensor response of exposure levels to populations, including estimates of populations affected at different levels (threshold reactions, incapacitation, various degrees of lethality). A web browser-based tool for rapid access to reference information, operational check lists and detailed facility information (also know as "target folders") was also developed. Both of these tools were used very successfully in the Westwind weapons of mass destruction field exercise and the Measured Response biological tabletop exercise (TTX). These prototype tools provided important capabilities to participants in these exercises. The functionality of these tools will be provided in BASIS on the ComCon; each tool may also be further developed for specialized application.

Field and Planning Exercise Support

BASIS prototype tools were deployed during the Westwind weapons of mass destruction exercise in Los Angeles in February, 1999. Initial BASIS activities at Westwind were in support of the Los Angeles County Operational Area Terrorism Early Warning group (TEW) in the Los Angeles County Emergency Operations Center (LACEOC). Analysis results and data from the BASIS prototype tools were used extensively in the LACEOC initially and very quickly spread to the field command post and Joint Operations Center (JOC). The BASIS team was invited to move to the JOC when most activities shifted there to provide a wide range of analyses that were prominently displayed and used in planning actions at the field command post and for tactical entries conducted as part of Westwind, as shown in the photo.

BASIS tools were deployed during the Westwind exercise.

Future Outlook

BASIS will move forward in FY00 and FY01 with development, validation, integration and testing activities that will culminate in the DDAP test in February, 2001. Based on successful completion of the DDAP, a decision will be made on deployment at a special event. Limited portions of BASIS may also be deployed at other special events in the interim, as needed.

The present BASIS architecture is constrained by available biological detection and identification technologies. As these technologies mature and become more capable and robust, the BASIS system can incorporate new technologies and capabilities through its "open architecture" approach. Thus, the future BASIS architecture may evolve to a system with distributed robust and reliable biological detection and identification technologies.

Field Tests

Westwind WMD Exercise, February 1999. Prototype BASIS information management and decision support tools were deployed with great success. The BASIS tools provided a unique capability in support of the Los Angeles County Emergency Operations Center and the Joint Operations Center. Analysis results were used extensively in planning and executing responses at all stages of the exercise.

Measure Response Bio TTX, June 1999. Prototype BASIS tools were introduced to a wide range of participants during this bio TTX. Specific analyses were provided as a part of the reference materials for the exercise.

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