

11B.1**Estimating Exposure Risk for Escaping Office Personnel.**

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The Ricin Technical Assistance Document prepared by ERT and U.S. EPA in response to the ricin attack on Capitol Hill on February 2, 2004, and subsequent analysis of the case revealed the need to develop quantitative methods to estimate risk resulting from exposure of rapidly evacuating (escaping) occupants to threat agents resuspended from surfaces in offices. The goal of this research effort is to determine quantitative estimates of risk to escaping occupants resulting from exposure to resuspended chemical and biological agents like ricin.

A study designed to elucidate the processes of exposure generated by escaping individuals was initiated using a specially designed articulated, heated, breathing manikin. The adult-sized manikin was upgraded to enable it to execute fast movements such as walking or running down or up stairs, which we believe are the most effective motions for stirring agents from the floor. Motion capability was achieved by attaching electric actuators to the manikin's limbs.

Three Aerodynamic Particle Sizers (APSs) were set up to measure particle size distribution and concentration in 1 s intervals. A sampling tube imbedded in the shoe allowed the particles to be sampled from the space under the foot in real time. The other APSs were set to measure particle concentration and distribution simultaneously from other areas along the manikin's body.

This study revealed that a particle-laden bolus is generated in the space opening during a step forward, moving rapidly from heel to toe. We found that a single footstep generated a bolus that typically lasted 3 seconds, with particle concentrations easily reaching 4000/cm³. Further investigation of particle transport is currently in progress, using PIV to investigate transport from under the foot space into the area above the foot space and further along the manikin's body into the breathing zone.

11B.2**Electrical Enrichment of Bioaerosols near Ground Level.**

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During fair weather, a population of particles has been observed near ground level with a size distribution skewed in favor of fine (<2.5 microns AD) particles, when compared to the particle population at two meters. Investigation of this layer, at a height (altitude) of 20 cm above ground level, shows that it can contain a disproportionately elevated concentration of viable bioaerosols. The ability to predict and better understand this phenomenon may be of significant importance in bioaerosol sampling and in children's health, and in bioaerosol source attribution.

Concurrent ambient aerosol samples were taken outdoors at heights of 20 cm above ground level and 200 cm above ground level. The samples were taken in a rural setting in Missouri, USA over a period of three years, using particle counters, APS, and a 6-stage viable impactor. Atmospheric electrical measurements were taken at 20 cm and 200 cm using an ion gauge and a static meter. Meteorological data were taken and weather patterns were recorded. Biological samples were cultured, enumerated, gram stained and classified. Particle counter data were reduced and compared to bioaerosol particulate matter concentrations.

Results show that the concentration ratio of fine particles near the ground during fair weather varies from approximately 200% to 1000% that of the population at two meters. The formation of this concentration of micron-sized biological particles is theorized to be due to levitation due to the typical net negative natural charge of the earth and the biological particles. The net negative natural charge present on populations of biological aerosols near the ground appears to be increased by accumulation of negative charges produced during the evaporation of water in the environment.

References: Yao, M. and Mainelis, G. Utilization of Natural Electrical Charges on Airborne Microorganisms for their Collection by Electrostatic Means, *JAS*, 37(4) 2006, Shalat, S., Liroy, Schmeelck, and Mainelis, Improving Estimation of Indoor Exposure to Inhalable Particles for Children in the First Year of Life, *J. Air and Waste Mgt. Assoc.* 57(8) 2007.

11B.3**Control-Volume Numerical Simulation of Bioaerosol Dispersion in the Atmospheric Surface Layer.**

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Most biological aerosol sampling devices are designed to collect particles in the 1-10 micrometer aerodynamic diameter (AD) size range. It has been suggested that this range be extended to include particles 0.5-30 micrometer AD for real-time bioaerosol detection (NRC 2005). When the method of detection depends on the mass of bioaerosol collected, increasing the upper limit of the particle size range may offer a net benefit. However, one may also consider that particles larger than 10 micrometer AD experience greater sedimentation, and thus would not warrant design of samplers for collection of particles in this range. In this study we estimate the change in the mass distribution function of a polydisperse aerosol over a range of distances down-wind from a release point. Particle transport is modeled by a two-dimensional convection-diffusion process including gravitational sedimentation. The transport of the aerosol release in the atmospheric surface layer is approximated with a control-volume numerical simulation. To characterize the wind speed and turbulent diffusivities we adopt the empirical relations proposed by Businger et al. (1971) which are based on Monin-Okhubov similarity theory. We acknowledge the presence of more sophisticated dispersion models; however, the current approach allows for a large number of initial distributions and flow conditions to be investigated, providing physical insight that would not be easily realized using more advanced models. Our data suggests that a significant mass fraction of particle sizes greater than 10 micrometer AD may transport distances on the order of several kilometers from a release point, and that a substantial increase in the aerosol mass collected may result if particles larger than 10 micrometer were included in the operating envelope of the sampling device.

National Research Council (2005). *Sensor Systems for Biological Agent Attacks: Protecting Buildings and Military Bases*. pp. 46-70.

Businger et al. (1971). *Journal of the Atmospheric Sciences*: 28: 181-189.

11B.4**Development of an Aerosol System for Uniformly Depositing Bacillus anthracis Spore Particles on Surfaces.**

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After the anthrax incidents in October 2001, several techniques used for sampling surfaces for biological agents were found to be inadequately validated, especially at low surface loadings. Therefore a system was developed to produce sample sets having targeted surface concentrations of dry biological agent simulant. A large test chamber was constructed and particles of dry agent simulant were aerosolized using a venturi aspirator. The aerosol was initially dispersed into the chamber at relatively high air concentrations, monitored in real time, and stirred using several fans. The concentration decay (stirred settling and dilution) was measured and when the targeted air concentration was reached, the sampling surfaces were uncovered and exposed to the settling particles until <99% of the particles had settled. Subsequently, the chamber was opened and the surfaces were sampled or removed for evaluation. Three types of surface samples could be exposed: agar plates, stainless steel, and carpet squares. The agar settle plates were used to estimate the true colony-forming-unit (CFU) surface concentration. The uniformity of surface loadings was limited by random deposition of small numbers of particles on the surfaces (Poisson distribution) and was characterized by how much greater the observed variability was than that predicted by Poisson statistics. The age of flow enhancer mixed with the spores appeared to affect the spores' ability to grow on the agar medium. The presence of spore agglomerates re-suspended by various sample handling activities in the chamber further increased the variability of deposited particles. When measures to reduce variability of the deposits were in place, the concentration of airborne particles measured using a particle spectrometer could be used to predict the mean surface concentration of viable spores within about +/- 20% at higher concentrations (>20 CFU/agar plate) and within about a factor of two at lower concentrations (1 to 10 CFU/agar plate).

14C.1

Can HEPA Filters Effectively Protect us from Viral

Aerosols? Brian Heimbuch, Jacqueline Hodge, Joseph Wander, Air Force Research Laboratory, MLQL, Tyndall Air Force Base; CHANG-YU WU, University of Florida.

High Efficiency Particulate Air (HEPA) filters are the primary technology used for particulate removal in individual and collective protection applications. HEPA filters are commonly thought to be impenetrable, but in fact they are only 99.97% efficient at collecting the most penetrating particle (~ 0.3 micrometer). While this is an impressive collection efficiency, HEPA filters may be penetrated by certain threats: Viruses are submicron in size and have small minimum infectious doses (MID). Thus, an appropriate viral challenge may yield penetration that will lead to infection of personnel. However, the overall particle size (agglomerated viruses and/or viruses attached to inert carriers) will determine the capture efficiency. Aerosolized viruses are commonly thought to exist as agglomerates, which would increase the particle size and consequently increase their capture efficiency. However, many of the threat agent viruses can be highly agglomerated and still exist as submicron particles. Furthermore the stability of aggregates is not well understood, and they may fragment during filtration. We have demonstrated in our laboratory that MS2 coli phage aerosols penetrate Carbon HEPA Aerosol Canisters and flat sheet HEPA material. A nebulized challenge of ~100,000 viable plaque forming units (PFU) per liter of air results in penetration of ~1-2 viable PFU. The penetration is linear over time, thus viral penetration is expected to occur in minutes following a viral challenge. AFRL is currently investigating the particle size distribution of the MS2 aerosol and our aim is to adjust the size distribution to measure its effect on penetration. Preliminary results indicate that 200-300-nm particles account for ~7.5% of the total number of particles. We are also evaluating the penetration characteristics of a mammalian virus, which may better represent the threat agent viruses. This study will expand our knowledge of the tactical threat posed by viral aerosols to HEPA filter systems.

14C.2

Re-Aerosolization During Doffing of Contaminated

Garments. JASON HILL, James Hanley, RTI International; James Hanzelka, U.S. Army Dugway Proving Ground.

An exploratory study was conducted to measure the re-aerosolization of particles during doffing of contaminated protective garments. The tests involved seven different garment systems utilizing either permeable or semi-permeable fabrics. Each test began with a system-level, human-subject aerosol exposure followed by controlled, assisted doffing. The tests used a non-toxic fluorescent aerosol simulant and were conducted in accordance with an approved human-use protocol.

The test method developed for the study employed two unique facilities: an aerosol exposure wind tunnel and an emissions quantification chamber. The exposure wind tunnel provided controlled contamination of the garment by the fluorescent aerosol. After exposure, the test participant entered the emission quantification chamber. The HEPA-filtered emissions chamber operated as a horizontal-flow cleanroom with focused exhaust flow to allow representative sampling of emissions as a function of time.

Re-aerosolization during the doffing process was measured by both filter-based and continuous samplers. Fluorometric analysis of the full-duration, filter-based samples provided data on total downstream emissions, fallout, and personal exposure of the doffers. Sequential filter samples obtained over subsets of the full test were compared to the continuous instruments (optical particle counter, Aerodynamic Particle Sizer, and photometer) to characterize emissions from portions of the doffing routine.

Results showed that the re-aerosolized mass was within measurement range of the various samplers. Evidence suggests dependencies of the re-aerosolized mass on the garment design (coverall vs. separate coat and trousers), doffing method (pull off vs. cut off), and permeability of garment fabric. Results for total fallout, the downstream airborne mass, the personal samplers on the doffers and the aerosol analyzers will be presented.

14C.3

Bioaerosol Detect-to-Warn Concept Based on Combined UV-fluorescence and background Aerosol Monitoring.

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One of the major challenges in real time detection of airborne biological threat agents (ABTA) is to distinguish the benign natural or man made bioaerosols from intentional release of ABTA.

No single available real-time detection technique gives a satisfactory criterion for detection. Thus the reliable biodection have to rely on more than one detection technology giving diverse information from aerosol background, concentration, size distribution and intrinsic fluorescence.

The most commonly used techniques are based on the measurement of intrinsic UV-fluorescence and the size of bioparticles. For any reliable alarming criteria the timely behaviour of the measured parameters compared to normal conditions is significant. This requires continuous measurement with fast response. Because extremely low concentrations of harmful bio agents can cause infection, sensitivity in terms of agent containing particles per liter air (ACPLA) is of a crucial importance.

In addition to the background bioaerosol, other aerosols (e.g. diesel soot) may yield also fluorescent response. The timely behaviour of this kind of aerosol typically resembles that of artificially spread bioaerosols. For that reason it is extremely difficult to distinguish using only software based algorithms.

Developed concept combines UV-fluorescence detection optics with a special background-aerosol detection system. The light source for exciting fluorescence is a CW semiconductor diode laser with 405 nm wavelength. The optical system detects both fluorescence and scattered light, indicating both the fluorescence tendency and concentration of particles. The practical system includes also a particle concentrator (CF = 500). The background aerosol detection system measures the concentration of particles causing typically false signals for UV-fluorescence detection unit. Characteristics for this technique are fast response time and sensitivity. It can be easily integrated into a robust biodetector for field operation. The practical realisation of the developed biodetector, as well as, the experimental results are presented.

14C.4

Rapid Detection and Identification of Airborne Microorganisms by a High-Throughput Atmospheric Pressure MALDI-MS.

BERK OKTEM, Appavu K. Sundaram, Vladimir M. Doroshenko; Science and Engineering Services Inc.

Rapid detection and identification of pathogenic microorganisms in contaminated food and environmental samples, especially after bio-terrorism related events, is critical for treatment of the infected patients as well as for proper decontamination efforts. Traditional microbiological methods to identify pathogens are time consuming, taking 24 to 48 hours for the confirmatory results to arrive. Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) has often been successfully used for rapid characterization of microorganisms. However due to introduction of the samples into vacuum, this often requires human intervention. In this work, we report development of a high-throughput atmospheric pressure (AP) MALDI-MS based assay with aerosol collection capability. It offers important benefits such as seamless integration of sample preparation with MS and fast overall analysis, on the order of minutes. It has an aerosol collector which collects airborne aerosols directly on the chemical processing plate. The input flow rate is 450 L/min, suitable for ambient sampling with low particle number concentration. For initial studies, a modified laboratory workstation is used for sample preparation with customized liquid handling. All sample processing is carried out on AP-MALDI target probe at 50C. To 2 mm of particle spot on the target surface 1 microliter of an agent specific extraction reagent is added. Digestion of proteins is then carried out in-situ using trypsin enzyme. Salts and other contaminants are then removed by washing with water. Matrix solution is then added. AP-MALDI MS and MS/MS spectra are acquired using an ion trap MS. MS/MS data is used to search in the publicly available database using MASCOT search engine to obtain species specific identification based on the peptide sequence. We have successfully tested this setup with AP-MALDI MS/MS based analysis to detect and identify simulants of threat agents such as *Bacillus globigii* spores, MS2 bacteriophage in different environments.

14C.5

Ambient aerosol measurements and field testing of a two wavelength fluorescence Excitation and Elastic Scatter bioaerosol system. V. SIVAPRAKASAM, A. Huston, H.B Lin, J. Eversole, J. Willey, Naval Research Laboratory, Washington DC

A bioaerosol sensor based on dual wavelength fluorescence excitation and multiple wavelength elastic scattering - has been developed and characterized for classifying micron-sized bioaerosols on the fly. We have been able to successfully classify different types of bioaerosols including proteins and bacteria (vegetative cells and spores) and distinguish them from several common interferents. We have studied a variety of simulants and interferents grown under varying conditions to characterize the performance of the instrument. Simulant and interferent aerosols were generated using several different techniques.

The UV-LIF instrument was tested during a two-week-long, blind field trial at the Edgewood, MD breeze tunnel. The instrument operated unattended while simulants and interferents were released at various random times during the test period. The results of the field trial will be highlighted in this presentation. We detected and correctly identified over 90 % of the simulant releases and had zero false alarms over the 2 week testing period. In order to gain a better understanding of the characteristics of aerosols in the ambient air, we conducted background aerosol measurements for over 2 months and have collected over 700 hours of data. These measurements will aid us in steering the algorithm development and understands the dynamics of the background aerosols. Some of the preliminary results from our bioaerosol classification algorithm effort will be presented.

14C.6

Development and Characterization of a Sulfur Mustard Aerosol CounterMeasures Laboratory. Jake McDonald, Yung-Sung Cheng, WAYLON WEBER, Yue Zhou, Lovelace Respiratory Research Institute.

A Sulfur Mustard (SM) synthesis, analysis and aerosol delivery laboratory was developed and characterized. The laboratory supports a COUNTERACT Center of Excellence that is aimed at developing therapies to counteract SM induced injury to eye, skin, and the respiratory tract. The laboratory provides the first known system for developing and studying SM vapors and aerosols directly. SM was synthesized and purified by published techniques. SM aerosol/vapor generation was conducted using a delivery system to best mimic potential human exposures. Characterization of vapor/droplets was conducted with a cryo focusing-thermal desorption gas chromatograph, providing analysis of both the gas and particle phase. Droplet size, which was modulated by a counter current heat exchanger, was characterized by impaction and differential mobility analysis. Initial findings and laboratory design will be reported.