



Transport and Dispersion of Biological Agents/Toxins

A response to a SCAPA/BWG Action Item

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November 2009



1.0 Background and Statement of Problem

U.S. Dept. of Energy (DOE) and Nuclear National Security Administration (NNSA) laboratories perform work for various agencies utilizing biohazardous microorganisms, viruses, and/or toxins. A subset of this work involves microbes, viruses, or toxins that are of concern from a potential for use as agents of bioterrorism or biowarfare (so-called “biological select agents and toxins” or “BSATs”) and are regulated by the Department of Health and Human Services (HHS) and the US Department of Agriculture (USDA) (7 CFR 331, 9 CFR 121, 42 CFR 73). Since 2001, DOE has mandated that DOE contractors and facilities comply with BSAT regulations (e.g., DOE Notice 450.7 and extensions). A more global consideration of infectious agents and toxins is now regulated in 10 CFR 851.

DOE O 151.1C (2005), and its accompanying biocontainment facility-specific guide, DOE G 151.1-5 (2007), address emergency management requirements that incorporate hazardous biological agents/toxins into site emergency management programs. The DOE comprehensive emergency management system consists of DOE-specific Hazardous Materials Program requirements added to and integrated with a Base Program consisting of other DOE directives and Federal regulations that govern the use and storage of Select Agents and Toxins, as mentioned above. The intended result is an integrated and comprehensive emergency management program that provides assurances of a timely and effective response to an onsite release, *observed* or *unobserved*, of a hazardous biological material.

Due to the wide range of agents and toxins, their diverse biohazardous properties (e.g., transmissibility), and uncertainties relating to their potential for release in emergency scenarios, the transport and dispersion of biological agents/toxins released from DOE/NNSA biosafety facilities was left an open subject in DOE G 151.1-5. In order to assist the DOE/NNSA Office of Emergency Management (NA-41) in providing policy and guidance to DOE/NNSA contractors and field elements, the DOE Subcommittee on Consequence Assessment and Protective Actions (SCAPA) Biosafety Working Group (BWG) was tasked with assessing the current state of knowledge related to laboratory-scale releases of etiological agents and toxins to the environment. In addressing this issue, several questions were posed:

- What models are available and appropriate for predictions, especially for lab size source terms, and not production quantities?
- What are the limits to the use of Gaussian models?
- What other modeling tools are available or being developed?
- Because a level of severity will likely not be available for defining a Protective Action Criterion (PAC), how will modeling results best be used?

This brief report describes the BWG approach and its assessment of the current status of analytic capabilities for modeling biological releases in the environment. In addition, several indoor modeling approaches are discussed related to the prediction of the source



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term from a release within a biocontainment facility. Note that a response to the last bulleted question, related to the interim use of modeling results, is still being developed.

2.0 Biological Agents and Toxins

Biological agents are living organisms or viruses capable of replicating after release. These agents include bacteria, fungi, and viruses. Bacteria can replicate in nature outside a human or animal host, while viruses require a permissive host to replicate; the tropism, or range of host tissues permissive for a specific virus to grow, can be narrow or broad. A subset of these agents and toxins that are of particular concern because of their potential malevolent use are referred to as BSATs and are regulated by federal regulations. In addition to specific agents and toxins, BSATs may also include laboratory samples, such as blood, saliva, semen, cell cultures or tissues that are, or may be infected with, such agents. [Such materials are by definition select agents, and if derived from humans, may also fall under Occupational Health and Safety Administration (OSHA) 29 CFR 1910, Section 1030, “Bloodborne Pathogens.”]

Toxins include peptides and secondary metabolites of living biological agents. These materials cannot reproduce and are generally considered toxic chemicals of biological origin [e.g., Centers for Disease Control and Prevention (CDC) guidance for safe handling of select agent toxins refers to OSHA regulations in 29 CFR 1910 § 1200, “Hazards Communications” and 29 CFR 1910 § 1450 “Toxic and Hazardous Substances”]. Moreover, toxins are frequently handled initially as powders which are highly dispersive and for which there are dispersion models. Toxin solutions, in contrast, more closely resemble pathogenic organism releases, where the potential for an evaporative source term is small, and a splash or other means for producing an aerosol is the much more likely airborne release mechanism.

Major sources of uncertainty for modeling bioagent releases into the environment are the data gaps regarding dose-response levels, particularly for the general population, environmental stability of the agent, and the decay rates for agent viability.

2.1 General Properties of Biological Aerosols

An aerosol is a suspension of fine solid particles or liquid droplets dispersed in a gas, usually air. A bioaerosol is an aerosol consisting of airborne biological particles or droplets. Generally a bioaerosol is generated as polydispersed droplets of particles of different sizes ranging from 0.5 μm to 30 μm in diameter. Particles over 100 μm in diameter are generally referred to as droplets. Particles from 1 to 2 μm can deposit deep into the alveoli of the lungs, while particles greater than 3.5 μm in diameter are trapped in the upper respiratory tract. Particles between 2 and 3.5 μm in diameter generally do both. Most pathogens target the alveoli, but there is evidence that primary infection for both Variola virus (i.e., smallpox) and *Yersinia pestis* bacilli (i.e., pneumonic plague) can occur in either the alveolar region or the upper respiratory tract.

Most bacteria range in size from 1 μm to 5 μm in diameter, and so cannot be carried on sub-micron diameter nanoparticles. On the other hand, viral particles and rickettsiae can be associated with sub-micron particles (Nicas et al 2005). This may be a

factor in the almost twice-higher laboratory-acquired infection (LAI) rate for viruses over bacteria and three times as high a rate for viruses over rickettsiae (Fleming and Hunt 2000). Large non-respirable particles ($d_{eq} \geq 50\mu\text{m}$) have a terminal settling velocity sufficiently high enough to cause most to settle out of room air close to the release point. Smaller airborne particles are removed by dry deposition processes by a first-order rate constant (Nicas et al, 2005). It should be noted that these smaller airborne pathogens are also biologically inactivated with a first-order rate constant which is agent-specific (Nicas et al 2005). Both dilution air and in-room High Efficiency Particulate Air (HEPA) filtration can remove viable agent particulates from the air with a calculable rate constant using Indoor Air Quality (IAQ) programs (see below and Bouilly et al 2005). Table 2-1 shows the particle type and its associated size range and settling velocity.

Particle Type	Size range (μm)	Settling velocity (fpm [*])
Droplet	100 - 400	59 - 498
Dust	10 - 100	0.59 - 59
Droplet nuclei	1 - 10	0.007 - 0.59
Droplet nuclei	0.1 - 1.0	0.00016 - 0.007

*feet per minute

Table 2.1: Particle type and associated size range and settling velocity (adapted from Herman 2007).

2.2 Properties of Biological Agents and Toxins in Bioaerosols

The following describe properties of biological agents and toxins that impact the analyses of biological releases, including the source term, the transport and dispersion of the materials in the environment, and assessment of health effects due to exposure to the materials.

2.2.1 Source Term Analysis

1. *Biological releases can easily be destroyed at the release point.* Unlike hazardous chemical or radioactive material, biological toxins and agents, due to their intrinsic fragility, can be completely inactivated using common decontamination agents such as household bleach.
2. *Airborne particle generation is activity-dependent.* Biological agents are normally handled in a research setting as liquid cultures whose accidental release as a droplet aerosol hazard can be due to a spill or to a release mechanism that entails significant energy (such as a centrifuge release). The particle size range for an agent release strongly depends on the activity involving the agents prior to the release or the activity that generated the release. Alternate types of releases will produce different spatial patterns of disease outbreak. For example, a study was performed with the opportunistic pathogen *Serratia marcescens* to determine the average particle size generated

during routine laboratory procedures and simulated laboratory incidents. The study demonstrated a wide range of particle size characteristics (Cf. Table 3 in Kenny and Sabel 1968). In particular, the results showed a direct relationship between energy imparted to the agent culture and the viable particle/ft³. Whereas, as expected, the energy imparted and average particle diameter are inversely related. In the high energy blender operation, >98% of the particles produced had a diameter of $\leq 5 \mu\text{m}$. In contrast, 80% of the viable particles aerosolized by low energy handling of lyophilized cultures were $> 5 \mu\text{m}$ in diameter.

3. *Personnel movement in the incident scene resuspends settled agent material post-release.* Biological aerosols move around buildings not only by air currents generated by ventilation [which can be modeled by many common Indoor Air Quality (IAQ) programs], but also by resuspension of settled materials. Because of the delayed onset of identifiable symptoms in a biological incident and the lack of characteristic signatures for biological agents (usually odorless and colorless), the area affected by a release may be greater due to the movement of contaminated and/or infected individuals who are unaware of the incident. Personnel movement in a contaminated area prior to incident recognition or as a result of emergency response to the incident (e.g., emergency operations, mitigation and restoration activities) may disturb settled material, spread the contamination, and resuspend biological materials into the air. It has been estimated that resuspension can extend the risk of infection from biological aerosols for hours and even days beyond an initial event when compared to allowing the particles to settle without disturbance (Sextro et al 2002; Lorenzetti 2009). This resuspension and its subsequent effects make the source term modeling process more complex.

2.2.2 Transport and Dispersion Analysis

1. *Non-sporulating biological agents and toxins are fragile.* Environmental releases must take into account strain- or toxin-specific loss of activity rates. Non-spore forms of biological agents are relatively fragile as compared to chemicals or radioactive materials, and inactivation occurs with exposure of the agents to drying, elevated temperature (i.e., $>60\text{-}100^\circ\text{C}$), and UV (solar) radiation. This makes biological agents hard to aerosolize and disperse without inactivating most or all of the agent material. Similarly, botulinum toxin, which is a BSAT and has a high oral toxicity ($\text{LD}_{50} = 1\text{-}2 \text{ ng/kg}$), is estimated to degrade at a rate of 1% to 4% per minute under standard laboratory room conditions.
2. *Natural (non-weaponized) biological agents in an airborne release are not sufficiently dispersive to infect a large population.* As an example, non-weaponized *B. anthracis* spores tend to clump together and settle rather than becoming airborne. It takes significant physical (e.g., freeze-drying/milling)

and chemical (e.g., silicate coating) treatment to render the properties of the spores such that they can be effectively transported in air. Modeling of airborne releases of biological agents must also take into account the reduction in concentration of the agent or toxin due to dilution after the release (expansion of the plume in large volumes of air).

3. *Agents can amplify post-release, while toxins cannot.* Biological agents can greatly amplify in quantity *in vivo*, by establishing an active infection in a permissive host (human and/or, for many agents, animal and insect vectors) or *in vitro*, given the appropriate growth conditions. Note that agents on fomites such as laboratory floors and benchtops have a characteristic decay half-life. In contrast, biological toxins are similar to radioactive material in that the quantity present in the environment is either the starting amount or a lesser amount due to decay.
4. *Biological agents are efficiently spread by live highly mobile vectors.* Once having established an active infection in a permissive host, biological agents can be transported by the host, creating a new source for release that could be highly mobile. Effective person-to-person transmission in nature beyond a distance of about one meter requires an energetic aerosol production mechanism such as sneezing. Nicas et al (2005) demonstrated that coughing and talking were not nearly as effective aerosol generators as sneezing or a highly mobile vector such as an arthropod (i.e., mosquito for West Nile Virus or *Plasmodium falciparum* (i.e., malaria) or insect (i.e., flea for bubonic plague) vector).
5. *Biological aerosols can change properties with time.* Airborne bioaerosols frequently change in diameter due to loss of water via evaporation (as much as 50%; Nicas et al, 2005)), changing their settling and dispersive properties. If the organisms are contained not in pure aqueous but in proteinaceous fluids (e.g., sputum, mucus, serum), evaporation will be much slower as these materials tend to retain water.
6. *Most infectious lab releases go unnoticed.* It has been estimated that up to 82 percent of all laboratory-acquired infections (LAIs) are not acquired via an identifiable release incident (Fleming and Hunt 2000). This requires a bio-surveillance approach to emergency preparedness and response which focuses on outbreak detection and inverse dispersion model analysis (cf. DOE G 151.1-5, Section 3.9 for a discussion of recognition of *observed* versus *unobserved* releases).

2.2.3 Health Effects Assessment

1. *Simple exposure to airborne infectious agents does not necessarily produce the disease in a host.* The mere presence of infectious organisms in the air is

generally insufficient to cause the disease. The properties of the bioaerosol must facilitate penetration into the lungs in sufficient quantity to achieve the inhalation infectious dose required to cause infection in the specific host (Fleming and Hunt, 2000). Lung deposition models of environmental biohazards have been used by several groups to guide workplace risk management (see Liao and Chen 2005 and Cho et al 2005). The effective infectious dose, or susceptibility, may also be influenced by factors such as the host's age, sex, pregnancy, immune system, and overall health status. The CDC and World Health Organization (WHO) have stratified the risk associated with particular agents into four risk groups (see Appendix A for more details) that take into account the following factors: (1) ID₅₀; (2) stability in the environment; (3) host range; and, (4) its endemic nature.

2. *Total dose is the critical parameter in assessing health consequences from exposures to infectious agent releases.* For an infectious agent release the important quantity to determine is not the concentration of airborne material at any time and location, but the total quantity of agent inhaled by each individual. This inhaled dose will determine the probability of active infection in a host when combined with the Infectious Dose (ID) for that agent. This is comparable to estimating internal deposition of radioactive particles from an airborne release.
3. *Biological agents cause delayed effects.* Toxic chemicals generally cause immediate effects, while radioactive materials can cause immediate or long-term effects depending on the dose received. Biological agents, as well as most biological toxins, cause delayed effects post-exposure (i.e., from hours for toxins to days or weeks for agents).

3.0 Indoor and Outdoor Modeling of Biological Agent Releases

At the outset of this project, CDC, the federal agency with responsibility for requiring incident response plans for using/storing select agents and that arguably has the greatest concentration and number of laboratories working with select agents, was contacted to obtain their views on modeling biological releases. The CDC provided the following guidance and direction on estimating source terms for the release of etiologic agents and toxins from laboratories: “The provisions adopted by each site will reflect the performance standards for biosafety, security, and incident response which are commensurate with the risks unique to the facility and the particular select agents or toxins used by the facility.”

Biological release “fate and transport” is quite complex and requires modeling capabilities that are still in the early stages of development and application. While many studies have investigated non-biological aerosol particle deposition, few have investigated bioaerosol either indoor or outdoor deposition including fungal spore deposition (Kanaani et al 2008). Few reports have attempted to validate predictions of active air contaminants (i.e., contaminants that undergo processes such as deposition, sorption and changes in size or density due to water uptake or loss) such as bioaerosols or particles. Authentic source term data for bioaerosols to aid in model validation is rare in the literature (see Taha et al 2006). Computer simulations of airplane cabin contamination using both computational fluid dynamics (CFD) (Lin et al 2005a and b) and deterministic models (see Mazumdar and Chen 2009) have been carried out, as well as dispersion models for possible bioterrorism attacks within enclosed spaces such as subway systems (Policastro and Gordon 1999).

These models of airflow inside buildings and subways have been developed using gaseous material assumptions and do not accurately incorporate the decrease in airborne concentration that results from deposition, or plate-out, of the toxic material on walls, ceilings, ventilation ducts, and other interior surfaces. Similarly, CFD models of the highly distorted flows and dispersion patterns created by complexes of buildings are just beginning to include the gravitational settling, deposition, and viability degradation effects of biological aerosols, and multiple building interactions [e.g., see the spread of *Bacillus anthracis* spores modeling by Sextro et al (2002) for indoor modeling and outdoor anthrax release modeling by Legrand et al (2009)].

A few reports have come out over the years where post-incident modeling has been performed to estimate the location and timing of the bioagent release (see Sellers et al 1979 and Hawker et al 1998). These are examples of vector-borne modeling. The most well-known study is based upon an accidental bioaerosol release from a weapons production facility in Sverdlovsk, Russia (see Meselson et al 1994) using gas phase estimation, and resulted in a fairly accurate model for the dispersion as assessed by illness onset over time. However, this is an anomaly as the agent released was a weaponized formulation of the pathogen specifically milled and chemically treated to enhance its dispersive properties. A more pertinent study undertaken to compare the

accuracy of two dispersion models in the prediction of distance traveled by airborne staphylococci demonstrated that neither the Eulerian Gaussian plume nor the Lagrangian particle-in-cell (PIC) dispersion model produced higher accuracies when compared to actual measured dispersion patterns, and that the values calculated by the two types of models were generally contradictory (Seedorf et al 2005). This may show that other vectors are at work in the dispersion processes that are not well known.

The next two sections address examples of modeling efforts related to internal airflow contaminant transport and external transport/dispersion of releases of biological agents to the environment, respectively. A preliminary assessment suggests that these models may be appropriate for predictions of lab size source terms and the transport/dispersion of the released biological materials in the environment.

3.1 Indoor Contaminant Airflow Models

A 1997 review by Argonne National Laboratory (ANL), Lawrence Berkeley National Laboratory (LBNL), Lawrence Livermore National Laboratory (LLNL) and Los Alamos National Laboratory (LANL) indicated that more than 50 interior building airflow models (ANL/EAD/TM-72) had been developed to date. Although most have not been applied to the problem of a bioaerosol release within research laboratories specifically, Emmerich in 2001 showed that CONTAM and COMIS are comparably accurate in multiple validation scenarios. It is important to note that all of the IAQ models tested by Emmerich performed best when the bioaerosol is modeled as a gas, which further emphasized the difficulty of modeling bioaerosols in general. Work performed at LBNL (Sextro et al 2002) modeling an indoor anthrax spore release assuming gaseous behavior utilized COMIS for the steady airflow rate calculations. An activity model was added that took into account exchanges between persons and building surfaces as well as spore deposition on “untracked” surfaces such as ducts or Heating Ventilation and Air Conditioning (HVAC) filters. This modeling assumed a constant 5 μm spore diameter and demonstrated that even 48 hours post-release, more than 90% of the released material remained in the building, predominantly on the floor surfaces where it would be subject to tracking and resuspension. None of the codes included tracking or resuspension modeling. It should also be noted that proper building flow data is needed to set up indoor flow models. This can be a major obstacle to using these models. Building ventilation / HVAC plans often do not reflect the actual circulation within buildings, and measurements need to be taken to characterize and validate these circulations for effective use of these models.

The following provides brief descriptions of the CONTAM and COMIS codes. A recent comparison of features between CONTAM and COMIS was provided at the 2009 SCAPA meeting and is available online (Lorenzetti 2009).

- A. **CONTAM** is a multi-zone (i.e., nodal) airflow and contaminant transport analysis program. It is a multi-zone indoor air quality and ventilation analysis hybrid, having both Gaussian and Lagrangian elements, computer code designed by the

National Institute for Standards and Technology (NIST) that is designed to determine the following:

- (1) airflows: infiltration, exfiltration, and room-to-room airflows in building systems driven by mechanical means, wind pressures acting on the exterior of the building, and buoyancy effects induced by the indoor and outdoor air temperature difference;
- (2) contaminant concentrations: the dispersal of airborne contaminants transported by these airflows; transformed by a variety of processes including chemical and radio-chemical transformation, adsorption and desorption to building materials, filtration, and deposition to building surfaces, etc.; and generated by a variety of source mechanisms; and/or,
- (3) personnel exposures: the predictions of exposure of occupants to airborne contaminants for eventual risk assessment.

CONTAM provides the capability to incorporate data from exterior airflow and pollutant transport models (e.g., CFD, plume and puff dispersion models) to utilize detailed ambient wind pressure and contaminant data fields. The program has an intuitive graphical user interface and is available as a free download for the Windows and Linux operating systems (NIST 2008).

- B. **COMIS** (Conjunction Of Multi-zone Infiltration Specialists) is a recent development in inter-zonal airflow modeling, with a modular structure that helps it simulate buildings more effectively than earlier multi-zone airflow models. It can be used as a stand-alone model with input and output features, or as an airflow module for thermal building simulation programs. It can also serve as a module library for other models. COMIS models the air flow and contaminant distributions in buildings. The program can simulate several key components influencing air flow: cracks, ducts, duct fittings, fans, flow controllers, large vertical openings (i.e., windows and doors), kitchen hoods, passive stacks, and "user-defined components."

COMIS allows the user to define schedules describing changes in the indoor temperature distribution, fan operations, pollutant concentration in each of the modeled zones, pollutant sources and sinks, opening of windows and doors, and weather data. This program has a large user base in Europe, but is no longer supported in the U.S.

3.2 Outdoor Airborne Dispersion Models

Although there are a large number of outdoor airborne dispersion models available (see FCM-I3-1999 for a federal directory of 64 different models), the following three models incorporate multiple phenomena which are important for biological release modeling. These phenomena include gravitational settling, deactivation of substance material, and a puff or single point release in time. The LODI model (to be discussed

below) appears to be the most advanced to date in incorporating these phenomena. None of the models have incorporated a time-dependent change in particle diameter characteristic of a bioaerosol. It should be also noted that these models are only as accurate as the airborne source terms used as input data.

1. **LODI** (Lagrangian Operational Dispersion Integrator) is a LLNL **NARAC** 3-D atmospheric dispersion model with the capability to simulate complex particle size distributions, wet deposition, bioaerosol gravitational settling, dry deposition (which is applicable to toxin powders), and deactivation of biological agents by ultraviolet (UV) radiation. LODI can simulate both instantaneous and continuous sources. Descriptions of the LODI model and its testing and evaluation have been published by LLNL (e.g., Nasstrom et al 2007; Leone et al., 2001). Dose-response models for bio agents are used with LODI prediction results in the NARAC system to predict effect (death, infection) probabilities. Due to the computational resources necessary, NARAC is typically not available for formal emergency planning hazard assessment (EPHA) purposes. However, for general emergency planning, consequence assessment and emergency response modeling the NARAC system is one of the most widely available tools at DOE sites across the country. NARAC has an emergency response service to incorporate air or ground contamination sampling after a release, and update model predictions during an incident response.
2. **CALPUFF** is a multi-layer, multi-species non-steady-state puff dispersion model that simulates the effects of time- and space-varying meteorological conditions on pollution transport, transformation and removal. CALPUFF can be applied on scales of tens to hundreds of kilometers. It includes algorithms for subgrid scale effects (e.g., terrain impingement), as well as, longer range effects (e.g., pollutant removal due to wet scavenging, dry deposition, chemical transformation) and also addresses visibility effects of particulate matter concentrations. CALPUFF does not contain a biological agent modeling component.
3. **SCIPUFF** (Second-order Closure Integrated PUFF Model) is a Lagrangian puff dispersion model that uses a collection of Gaussian puffs to predict three-dimensional, time-dependent pollutant concentrations. SCIPUFF is a key module in the Defense Threat Reduction Agency (DTRA) **HPAC** (Hazard Prediction and Assessment Capability) tool (note: the HPAC tool suite is in the process of being replaced by web-based functionality in the Joint Effects Model, JEM). In addition to the average concentration value, SCIPUFF provides a prediction of the statistical variance in the concentration field resulting from the random fluctuations in the wind field. SCIPUFF has been developed with a flexible interface, to describe many types of source geometries and material properties. Solid particles, liquid droplets, and gaseous materials are represented, with both primary and secondary evaporation mechanisms that produce vapor puffs as the droplets evaporate in the air or after deposition on the ground. A pull-down menu for specific bio-agents is available. HPAC plots hazard contours as either

- integrated dosage or concentration values and as text labels listing human effects (casualties and PAR). Human effects are given as probability of infection and probability of mortality. It has been suggested that HPAC/SCIPUFF is well suited for the rapid prediction of the impacts of an emergency event for first responders (Sohn et al 2004) because of its low cost, speed, and easy to use interface.
4. **MPR** (Maximum Possible Risk) Modeling. The MPR methodology (Schutz et al 2008; Cohen et al 2008) uses a simple release model to estimate the concentration in air near the release point by limiting the total volume (e.g., half cone) that can contain the released materials. The MPR algorithm is a conservative approximation that is used because of possible Gaussian model limitations for estimating release concentrations near the source (<100m). The model is used in risk assessments of high containment laboratories to determine upper limits on the possibility of an aerosol release of pathogens such as anthrax spores from a biological research laboratory. Although the DOE methodology is **not based on risk assessment** for emergency planning (cf. DOE G 151.1-1A), the simple model can be useful for obtaining estimates of concentrations of biological materials for close-in lab scale releases, where the use of the Gaussian model may be questionable.

4.0 Biological Agent or Toxin Release Scenarios

There are four broad categories of biohazardous material releases that would precipitate an Operational Emergency (OE) as defined in DOE O 151.1C (2005). The scenarios are given below in the order of most to least likely based on historical evidence of past laboratory releases of biological agents or toxins.

1. *LAI of unknown source detected by disease diagnosis.* Given the data available in the literature regarding laboratory work on pathogens, the most likely scenario that would trigger an OE is an acquired illness not preceded by an identifiable release incident (see Fleming and Hunt 2000). Epidemiological studies have traced these infections to various types of unrecognized releases including *C. burnetii*-contaminated laundry (U.S. Dept. of Public Health and Human Services et al 2007) and insufficiently rigorous viral inactivation protocols (LAIs of SARS followed by secondary transmissions to family members and tertiary infections to hospital personnel; CDC 2004). Outbreak detection modeling to identify the location, quantity and timing of release requires bio-surveillance data combined with an inverse dispersion model. Most inverse models use Bayesian methodology (see Jiang 2007 and Wong et al 2005 as examples) which is based upon the statistical significance of any illness observation above anticipated background illness. These models often incorporate component models within (e.g., dispersion, infection, disease and behavior), and data-source models (see Buckeridge et al 2004 as an example). The most cited inverse dispersion models are the BARD (Bayesian Aerosol Release Detector) (Hogan et al 2007) and the PANDA (Population-wide ANomaly Detection and Assessment) models (Cooper et al 2004). Given the recent nature of this work, there is a scarcity of data available for validating this class of models.
2. *Overt spillage within the laboratory containment space.* There were a number of LAIs caused by laboratory spills reported in the literature over the past few decades (see Fleming and Hunt 2000 for a summary). Although IAQ modeling of particulates as described above is the approach most likely to generate accurate values for inhalational exposure in this scenario, the models are still limited by a lack of accurate source terms for the amount of bioaerosol actually generated under different laboratory conditions (see discussion above).
3. *Vector spread of biological agent.* There are only a few studies that have looked at vector spread of animal and/or plant pathogens in nature (see Sellers et al 1979 and Hawker et al 1998 as well as the review by Davis 1987). There has only been one recent outdoor release of a zoonotic or plant pathogen as a result of an inadvertent vector-borne laboratory release. A recent accidental release of three *Yersinia pestis*-infected mice from a containment laboratory at the University of Medicine and Dentistry of New Jersey (UMDNJ) did not appear to have any environmental effects on the surrounding area.

4. *Release of a biological agent to the environment.* Events to be considered for analysis of biological releases to the environment include accidents, natural phenomena, external events (e.g., aircraft crash), and malevolent events [cf. DOE G 151.1-5 (2007), Section 4.2.3]. The catastrophic breach in the building integrity of containment laboratories is analyzed in facility environmental assessments or environmental impact statements (so-called ‘maximum credible events’). Release of agents or toxins during transport could also constitute a release to the environment. The recent Federal Working Group on Strengthening the Biosecurity of the United States pointed out in their final draft report (not yet released to the public) that in over twenty years of transporting BSATs within the U.S. there have been only two cases of lost material reported and no accidental releases. In England in August through September of 2007 an accidental release of FMD virus via leaking aging drainage pipes between the laboratory building and the nearby decontamination plant resulted in an extensive outbreak of FMD in the surrounding animal population and significant economic damage to the cattle industry in England for an extended length of time (HSE Report 2007).

5.0 Specific Recommendations to NA-41 from the BWG

The SCAPA BWG presents the following recommendations to NA-41 related to bioaerosol source term analysis and transport/dispersion models:

1. Given the present lack of more accurate source terms and bioaerosol-specific dispersion models, the IAQ model best presently suited for aqueous culture BSATs is CONTAM, as it allows for decay parameter inclusion, removal via filtration, deposition and adsorption, non-tracer contaminant incorporation and occupant-generated contamination. More research is necessary to demonstrate that CONTAM can accurately model indoor laboratory release scenarios that might be anticipated at DOE/NNSA facilities, and assist in determining potential indoor distribution and outdoor release of bioaerosols. However, NIST has confirmed that CONTAM can model particle filters and even combine various filter models, which would allow its use in predicting contaminant movement in highly engineered containment laboratories (W. Dols personal communication).
2. The outdoor release model which encompasses the most input parameters needed for bioaerosol modeling appears to be LODI. The use of HPAC/SCIPUFF (and its successor, JEM, when available) should also be considered for emergency planning. The Savannah River Site has published a report describing their experiences using this package (WSRC 2004), as has a consortium of European agencies (Pedersen et al, 2007). Additional work is necessary to research, develop, integrate and test appropriate source term models for outdoor bioagent dispersion models. Research should continue in order to fill existing data gaps in source terms, bioaerosol properties, bio-agent degradation rates in the environment and civilian dose-response models. It is important to note that, as for indoor models, studies have not extensively tested outdoor release models for validity in modeling bioaerosols (see descriptions above).
3. Given the proportion of LAIs which are not preceded by overt releases within containment, the DOE Office of Health, Safety, and Security (HSS) should strongly encourage the development of an active relationship between the Occupational Health Departments (OHDs) of those national labs with biocontainment laboratories and local public health departments, possibly via a revision of 10 CFR 851. This should include the development of a strong relationship between the laboratory OHDs and local emergency rooms to facilitate the exchange of expertise and information and enhance area bio-surveillance.

This recommendation is consistent with DOE G 151.1-5, Section 3.9: "...*unobserved* releases (e.g., unreported infected host, contaminated vectors) could remain undetected for a substantial period following the actual event at the facility. Recognition of these events can occur as the result of indirect detection of the release, when infected receptor(s) present symptoms of the disease. An active, ongoing medical surveillance program within the DOE/NNSA community and in

the local community can provide an essential detection capability for identifying a possible release from the facility. As with *observed releases*, early recognition of an actual or potential unobserved release of a biological agent is essential for emergency response measures to be most effective.”

4. National Laboratory BSAT facility biosafety manuals should include detailed information relating to local resources for post-incident environmental testing of laboratories and gaseous decontamination protocols if deemed necessary. These recommendations are consistent with the recent planning guidance provided by the U.S. Dept. of Homeland Security (DHS) and the Environmental Protection Agency (EPA) (EPA 2009).
5. National Laboratory incident response planning should include a consideration of the four release scenarios described in Section 4 of this report where appropriate to the biological agents and toxins used. Use of the recommended transport and dispersion models for indoor and outdoor releases may permit the estimation of infectious agent or toxin releases from DOE/NNSA facilities and assist emergency planners and incident responders to plan and take appropriate protective actions and recovery activities in the event of a release.



6.0 References

7 CFR 331, 9 CFR 121, “Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins; Final Rule”, March 18, 2005.

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Appendix A

Biological Agent Hazard Stratification– Common Approaches

WHO Risk Group. The World Health Organization (WHO) periodically updates the “Laboratory biosafety manual” (now in its 3rd edition, 2004). This manual provides two tables in the General Principles section that summarize current practices of biosafety – classification of the agents by risk group, and appropriate biocontainment practices, or biosafety levels, which factor in the types of operations and the risk group of the agents. These are provided directly from the manual in Tables 1 and 2 below. It should be noted that there is not necessarily a one-to-one correspondence between risk group and recommended biosafety level, as the operations and practices, as well as quantities of material may be factored in to reduce the recommended biosafety level for the work to be performed.

Table 1. Classification of infective microorganisms by risk group

Risk Group 1 (*no or low individual and community risk*)

A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 (*moderate individual risk, low community risk*)

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (*high individual risk, low community risk*)

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (*high individual and community risk*)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Table 2. Relation of risk groups to biosafety levels, practices and equipment

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

CDC BMBL. The U.S. Centers for Disease Control and Prevention (CDC) periodically update a comparable manual, entitled “Biosafety in Microbiological and Biomedical Laboratories” (BMBL). This document is incorporated by reference in the various select agent regulations mentioned earlier in this report, and during inspections to assess compliance. Section II of the BMBL contains a more detailed discussion of biological risk assessment, and reflects U.S. practice, considering “Hazardous characteristics of an agent” that includes a table of risk groups much like that found in the WHO manual, but also recommends consideration of probable routes of transmission of laboratory infections, infectious dose, stability in the environment, host range, and (its) endemic nature.” Additionally, consideration of reports of laboratory acquired infection (LAI) is recommended, to review lessons learned that may assist in establishing better controls. Beyond the inherent risks of the agents, biological risk assessment as described in the BMBL also considers the risks of the laboratory procedures, including the use of sharps, potential for generating aerosols, particularly of respirable particles, splash hazards. Experience of the staff, the complexity of the procedures to be performed, personal protective equipment, available laboratory equipment, and adequate training may all be factors in safe execution of the work, and should be factored into the recommended biocontainment level.

Table 3. Concentration and Particle Characteristics of *S. marcescens* aerosols created during several laboratory procedures and incidents (adapted from Kenny and Sabel 1968).

Operation	Mean viable particles/ft ³ of air sampled	Count median diameter μm
Harvesting infected egg ^a	22.0	3.5 \pm 1.6
Mixing culture with pipette	6.6	2.3 \pm 1.0
Use of blender:		
Top on blender in operation	119.6	1.9 \pm 0.7
Top removed after operation	1,500.1	1.7 \pm 0.5
Mixing culture with mechanical mixer:		
For 15 sec	0.0	0.0 ^b
Overflow	9.4	4.8 \pm 1.9
Use of centrifuge:		
No spilled material	0.0	0.0
Material spilled on rotor	1.9	4.0 \pm 1.8
Use of sonicator	6.3	4.8 \pm 1.6
Opening lyophilized cultures suspended in: 3% lactose	134.7	10.0 \pm 4.3
3% lactose plus mother liquor	32.6	8.0 \pm 3.4
Dropping lyophilized culture suspended in 3% lactose	4,838.7	10.0 \pm 4.8
Dropping infected egg ^c	85.2	3.0 \pm 1.3
Dropping flask culture	1,551.0	3.5 \pm 2.0
Spilling culture from pipette	2.7	4.9 \pm 2.6

^a Approximately 1.4×10^{11} viable cells/egg

^b A total of 4 viable particles sampled in 10 trials.

^c Approximately 9.8×10^{10} viable cells/egg.