Environmental Assessment for *Bacillus subtilis* Particles to Challenge Bio-Detection Sensors in Subway Stations

Prepared for Department of Homeland Security Science and Technology Directorate

January 12, 2012
Version 15

EXECUTIVE SUMMARY

This Environmental Assessment (EA) documents the analysis of the potential effects of a proposal by the Department of Homeland Security (DHS) Science & Technology Directorate (S&T) to conduct tests and experiments involving the release of low concentrations of particles at certain stations within the Massachusetts Bay Transit Authority's (MBTA's) Subway 'T' System. No construction, permanent land disturbance, or land use changes would occur with implementation of the Proposed Action or the Alternatives.

DHS S&T has been developing technologies and sensors needed to rapidly detect a potential biological attack on the Nation's transportation infrastructure in order to minimize public exposure and strengthen security. To validate the performance of the technologies, it is necessary to perform field tests in a real-world environment. Subway systems provide one of the most challenging and harsh indoor settings that sensors of this nature would be exposed to in real-world deployment, due to the temperature and humidity extremes that often characterize these types of indoor environments. In order to understand the true detection capabilities of the biological sensor networks, challenge tests with a material must be performed. Since a portion of the technologies rely on the detection of genetic or proteinaceous materials to positively identify a particular threat agent, the simulant must be of biological origin. *Bacillus subtilis,* or *B. subtilis,* a soil bacterium which is not pathogenic to humans, has been studied extensively for human, animal, and environmental safety, and has ultimately been approved by the United States Environmental Protection Agency (EPA) for day of harvest use on produce as a bio-fungicide. For these reasons *B. subtilis* has been chosen to serve as the particulate material for the proposed tests.

There are four action alternatives presented in this assessment to evaluate tradeoffs in test procedures, which would either fully or partially meet the needs of DHS S&T; additionally there is a no action alternative, which would involve no particulate releases:

The first alternative is to conduct an aerosol release of known quantities of *B. subtilis* within the subway system to demonstrate a positive detection of the material by the sensor network installed in several underground stations. These studies, to be performed at peak operational capacity for trains and passengers, are designed to most closely simulate the conditions that would likely exist in the event of a true bio-terrorist attack.

The second alternative is to conduct an aerosol release of nonviable (killed) *B. subtilis* particles for testing the sensors during revenue hours. The killed material, because it is no longer an active biological substance, is considered as a particulate or dust nuisance. This alternative would alter the test material, but not the test conditions or the test release as described in Alternative 1.

The third alternative is to conduct an aerosol release of nonviable (killed) *B. subtilis* spores for testing sensors during non-revenue hours for the subway. The trains would be operated to mimic a peak schedule, but no passengers would be present in the stations. This alternative would alter the test conditions, but not the test material or release as described in Alternative 2.

The fourth alternative would be the direct injection of viable *B. subtilis* spore aerosol into a single sensor during operational hours for the subway, and capture all of the test material within the sensor such that it does not enter the subway station environment at all. This alternative would not alter the proposed test material, but would alter the test release and conditions as described in Alternative 1.

Due to the potential human health and safety risks posed by the presence of sensitive populations, to include immune-compromised riders during operational hours, the aerosolization of viable spores to challenge the biosensor system, as outlined in Alternative 1, is not recommended. Implementing the use of nonviable material, as outlined in Alternatives 2 and 3, will ensure the health and safety of all subway riders including sensitive populations without compromising the results of the testing activities. Alternative 4 presents no potential adverse human health or safety impacts; however the procedure as outlined does not fulfill the purpose of the aerosol tests.

The indirect environmental effects caused by the potential exposure of terrestrial wildlife by movement of the material out of subway tunnels and into the open air were also evaluated. The environmental consequences posed by any of the alternatives as outlined will not have an adverse effect on terrestrial wildlife.

In accordance with Executive Order 12898, analysis of the environmental effects must also include effects on minority communities and low-income communities, when such analysis is required by the National Environmental Policy Act of 1969 (NEPA), 42 U.S.C. section 4321 et esq. Overall, populations using the subway as well as those living in and around the effected subway stations do not disproportionally represent minority and low-income populations; implementation of alternatives has no adverse impact on resources, human health or the environment.

As a commercial biofungicide, the *B. subtilis* test material has undergone rigorous studies to evaluate the potential health effects and safety of the material for the general public, workers and environments surrounding the commercial use, and no adverse health effects from low level exposure to *B. subtilis* in healthy populations have been documented. The quantity of material proposed for these tests is well below the dose rates for the toxicology testing of these biofungicides and the reported results provide a conservative comparison. Therefore, Alternative 1 would be the preferred test condition to provide the most realistic challenge to the system. However, taking into account any health-

related concerns over the potential presence of immune-compromised individuals, young children or elderly, or asthma sufferers who are sensitive to dust particle loads, the use of the nonviable material during non-revenue hours described in Alternative 3 is the Proposed Action for these tests.

DHS S&T provided two opportunities for the public to comment on the Proposed Action: a public forum, held in Cambridge, MA on May 16, 2012; and an online posting of the EA with an email address open for a 6-week period from April 30 to June 15, 2012. Public input submitted through these methods did not result in any changes to the conclusions of the EA or the Proposed Action.

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Section 1. Purpose and Need of the Proposed Action

A strategic goal of the United States (US) Department of Homeland Security (DHS) is to prevent, detect, and protect against biological attacks.[1] Protecting our critical infrastructure from the effects of biological weapons attacks is a key element to achieving this goal. Early warning systems and rapid detection of a biological agent attack will enable rapid responses to prevent the loss of life, psychological trauma, and illness, and to contain the spread of potentially contagious diseases. Information collected during an attack will support response and restoration operations to protect public health and welfare and minimize the economic impact of a biological attack.

The Science and Technology (S&T) Directorate within DHS collaborates with and leverages the scientific, engineering, and technological resources of the United States in developing new technological tools to execute the DHS mission. DHS S&T has been researching and developing technologies and sensors needed to detect, mitigate, and recover from possible biological attacks on the Nation's infrastructure. A key aspect to an effective response to a biological attack is the rapid detection, identification, and characterization of an event. Several biological detection sensors that can be deployed and operated in large indoor areas, such as subways, have been developed by DHS S&T to meet the need for rapid detection and identification of a biological attack. Previous research and modeling of airflows, particle transport dynamics, and assessment of normal background conditions in a subway transportation system have provided the data required to devise scenarios to estimate the reliability of these sensors for detection and identification of a biological attack in an operating subway environment.[2,3] However, to validate the detection performance of a sensor network in its entirety, real-time in-situ challenge tests using known quantities of a well-characterized organism within the subway system is necessary to demonstrate positive detection of the material by the sensor network. Without meaningful validation under realistic threat scenarios, the actual measure of protection these systems will provide in the operational environment is academic rather than demonstrated.

The purpose of this Environmental Assessment is to determine the appropriate material and conditions to safely conduct tests of a network of biological detection sensors installed in an operational subway system. DHS S&T proposes to test the detection performance of the sensors using small quantities of *Bacillus subtilis* as a particulate material. This assessment will examine the tradeoffs between several subway systems in the nation, the use of live or killed *B. subtilis*, and testing during revenue or non-revenue hours. DHS S&T has partnered with the Massachusetts Bay Transportation Authority (MBTA), Massachusetts Department of Public Health (MDPH), and Massachusetts Emergency Management Agency (MEMA) to help define the impact of these tradeoffs on the public as well as to help plan the immediate response and remediation actions to a biological attack in order to protect public health and

welfare. The proposed testing action will determine the accuracy and sensitivity of the rapid biological detection network, as well as the long term durability of operating such a network.

This testing will support the transition into operational use of biological detection sensors, as well as aid in validating the outputs of predictive sensor performance models. If these tests show a successful performance of the biological detection network, this data will be used by DHS agencies, such as the Transportation Security Administration (TSA) and Office of Health Affairs (OHA), among others, to inform future biological detection system acquisition programs. If, however, the results from the test show that the performance of the network is not adequate to provide reliable detection of an event, then the data from the tests will be used to inform further sensor development efforts to overcome these challenges.

Section 2. Test Alternatives to Meet the Need

This section will detail the range of test alternatives, as well as a no-action alternative, examined in order to determine the Proposed Action. The analysis of the alternatives and no action alternative is being conducted in accordance with the National Environmental Policy Act (NEPA) as outlined in 40 CFR Parts 1500-1508 and DHS's implementing regulation Management Directive 023-01, Environmental Planning Program.

2.1 Alternative 1

The first alternative is to aerosolize known quantities of a well-characterized, non-pathogenic, microbiological organism within the subway system to demonstrate positive detection of the material by the sensor network installed in several underground stations. Activity in the subway stations should be at peak operational capacity for trains and passengers to most closely simulate the conditions that would likely exist in the event of a true bio-terrorist attack.

2.1.1 Test Conditions

The optimum condition for executing a bio-terror attack would be during rush hour, when a large number of riders are in the stations and trains are running at peak number and speed. The transport of aerosols is largely driven by the motion of trains in the system, and thus the most accurate reflection of material transport would involve testing when trains are at maximum operational capacity.

Train induced airflow patterns developed for use in the design of subway station cooling and heating capacities were initially used for estimating transport of contaminates in the subway system air.[4] Initial field tests were then completed

to further refine this airflow model using known quantities of gas and observing the airflow as trains passed through the environment. The use of the refined model allows for the development of specifications for detection and warning systems against biological particulates, and the determination of deployment and countermeasure strategies. However further field tests are a necessary partner to validate the models and provide real-time data to continue model refinement to assure confidence in future computational outputs.

2.1.2 Test Material

The particulate simulant test material is the spore form of a non-pathogenic gram positive bacterium named *Bacillus subtilis*, frequently referred to as *B. subtilis*. *B. subtilis* is a ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue that is not considered toxic or pathogenic to humans, animals, or plants.[5] *B. subtilis* produces a variety of enzymes that enable it to degrade a range of natural substrates and contribute to nutrient cycling. *Bacillus* bacteria naturally produce a spore coating that allows them to endure extreme conditions of heat and desiccation in the environment. The *subtilis* species is abundant in nature, well defined, with a number of strains currently used in multiple commercial applications including: specialty manufacturing, agricultural biofungicide, and human and animal food supplements and probiotics.[6,7, 8,9,10,]

There are four strains of *B. subtilis* spores registered by the US Environmental Protection Agency (EPA) for use as a biofungicide on food crops, in addition to ornamental (flowering and foliage), greenhouse, and home garden plants.[5] The application of the biofungicide product controls fungal and bacterial outbreaks which would otherwise attack and degrade or destroy the crops or plants. These products present a commercially available source of well-researched *B. subtilis* spores for use as a test material.

Beyond its agricultural use, *B. subtilis* has gained popularity as a probiotic and food supplement and is in the process of being examined in the context of human consumption under The Joint Food and Agriculture Organization of the United Nations and the World Health Organizations approach for probiotics in food.[9] In addition to its use as a human food supplement, a commercially marketed *B. subtilis* product is approved in the US, European Union, Brazil, many countries of Asia (including China, Thailand and Japan etc), and Mexico as a direct feed microbiological to benefit poultry and livestock growth and health.[7] The *B. subtilis* products are found to be effective competitive exclusion agents for use in poultry to control avian pathogens and improve feed conversion and average daily weight gain.[7] Fermentation products of *B. subtilis* can also be found as a feed additive in organic pet foods sold in the US.[10]

The many commercial uses of *B. subtilis* have also led to the development and design of assays to detect and identify these organisms for quality control purposes. These well-established assay designs can be easily utilized by the

sensor technologies being tested as the focus of this study. Taken together, this material provides a safe, rapid, accurate, and cost-effective method for challenging the sensors being developed by DHS S&T for the rapid detection of biological threat agents.

2.1.3 Test Release

An in-situ challenge test within a subway station would involve an aerosol release of approximately 10-50 grams (0.4-1.8 ounces) of a powdered formulation containing no more than 25-30% *B. subtilis* spores. Laboratory culture analysis of the material yields approximately 3×10^{10} colony forming units per gram (cfu/g) of the material. The remaining 70-75% of the material is comprised of an inert filler which aides in assuring an even and fluid dispersion of the challenge material. The material will be released using a dry air pump to aerosolize the test material in a single burst. This release technique will ensure that the size of the particles released are only a few microns in diameter, and that the tests would reasonably replicate a bioterrorist release scenerio. The challenge material will be pre-filled into the aersolization unit in a laboratory. The product is safe to handle on the lab bench top, as outlined for organisms which are identified to require Biosafety Level- 1 (BSL-1) controls.[11] The filled aerosolization units will be placed in a shatter resistant, leak-proof sealed secondary containment for transport to the test site.

The biological sensor network will collect data of the aerosol concentrations and response of automated aerosol sampling and detection devices to the test organism. The biological sensor systems will operate continuously and may be tested up to four times per month over a one year time period. Sensors will remain operating in the stations between challenge tests to gain a better understanding of the effect that background particulate matter in the subway evironment has on sensor operation.

2.2 Alternative 2

The second alternative is to conduct an aerosol release of nonviable (killed) *B. subtilis* spores for testing the sensors during revenue hours. This alternative would alter the test material, but not the test conditions or the test release as described in Alternative 1.

Test material: The *B. subtilis* spores would be made nonviable via gamma-ray irradiation of the live *B. subtilis* in the laboratory prior to the test event. The composition of the test material would be identical in make-up with the exception that the nonviable *B. subtilis* spores would not be capable of germination and growth since they have been rendered dead, and thus would be simply a particulate material rather than a living biological preparation. Thus, the particulates would be simply a dust nuisance, rather than a material which would

have any health or allergic risk. The sensors being tested can detect both live and killed spore material, so the killed material will be suitable for the challenge tests.

The irradiation methods which will be used to kill the *B. subtilis* spores are identical to the irradiation methods which are used to sterilize food products. The Food and Drug Administration (FDA), US Department of Agriculture (USDA), Centers for Disease Control and Prevention (CDC), and World Health Organization (WHO) approve food irradiation techniques for a number of foods, including herbs and spices, fresh fruits and vegetables, wheat, flour, pork, poultry, and red meat. The safety of irradiated food has been well researched and documented to kill harmful bacteria and control food spoilage. Irradiation of the material does not make it radioactive.[12] Kill curves, which are used to determine the viability of the microorganism as a function of irradiation treatment times, are well established for *Bacillus* spores. Prior to use, samples of the irradiated *B. subtilis* would be tested for growth in ideal laboratory conditions to ensure that the spores have been killed. A Kill Certificate will accompany the material, and only *B. subtilis* that have been certified as killed will be used in testing.

2.3 Alternative 3

The third alternative is to conduct an aerosol release of nonviable (killed) *B. subtilis* spores for testing sensors during non-revenue hours for the subway. The trains would be operated to mimic a peak schedule, but no passengers would be in the stations. This alternative would alter the test conditions, but not the test material or release as described in Alternative 2. Using the killed *B. subtilis* would also ensure that no biological particles introduced through this test would remain as a living material in the subway system when revenue hours recommence.

Test conditions: Trains would be operated to provide the transport of aerosols; however, testing during non-revenue hours would eliminate the effects caused by movement of passengers in, through, and out of the stations.

2.4 Alternative 4

The fourth alternative would be the direct injection of *B. subtilis* spore aerosol into a single sensor as a “spike test” during operational hours for the subway, and capture of all test material within the sensor such that it does not enter the subway station environment at all. This “spike test” could involve the use of a containment device around the installed sensors and/or the use a direct insertion method into the air sampling inlet. This alternative would not alter the proposed test material, but would alter the test release and conditions as described in Alternative 1.

Test conditions: While the test conditions in the subway will not be altered to implement this alternative, the use of a direct challenge to a single sensor eliminates the influence of the test conditions on the test. The alteration of the test release method to a direct challenge eliminates the effects of air movement within the subway system. However, many models show that air movements within the subway are very important factors in how material is moved, and removing this test condition will remove the ability of the challenge tests to account for this important factor in the overall detection performance assessment of the sensor network.

Test release: The release of a significantly smaller quantity of the aerosol challenge test spores would be made directly to a single sensor, rather than into the subway station. To ensure that all of the challenge material enters the sensor, the output of the direct feed aerosol device will have a lower air-flow rate than the air intake of the sensor device. Such procedures would ensure the material is drawn into the sensor and not into the subway station. The sensor will then pull in both the test release as well as background air from the subway station. In this way, this alternative will challenge the sensor with finding a positive detection in the presence of true subway background materials, a test which cannot be easily repeated in a laboratory environment.

2.5 No-Action Alternative

The no-action alternative is to continue to develop the dispersion model using current methodologies and challenge the sensor technologies only in a laboratory setting. The sensor network may be operated in the subway stations to monitor for background interferents and to further understand the durability and reliability of the sensors; however no in-situ challenge testing would be conducted in the subway.

The no action alternative would eliminate conducting in-situ challenge tests of a biological sensor network system during a year-long deployment to determine the accuracy, sensitivity, and durability of the sensors to detect a credible biological aerosol threat, as well as to validate the outputs of predictive sensor performance models within the subway system. As a piece of life safety equipment, similar to a fire detection system, these systems cannot be deployed and operated with confidence unless tests are performed to ensure that the protective capabilities are verified. Therefore, this alternative does not meet the needs of the development effort and test.

Section 3. Affected Environment

Several subway systems across the country were considered to host the proposed pilot test. The criteria for an optimal location for the study included: a large system that has subterranean stations; a geographical location that exhibits environmental extremes, with a particular focus on temperature and humidity; a system which has non-revenue hours for Alternative 3 test conditions; and a system in which research studies on the modeling of airflows, particle transport dynamics, and assessments of normal background conditions have been previously carried out.

Preliminary discussions were held with a few subway systems, and the Massachusetts Bay Transit Authority (MBTA) was considered to be an optimal location due to the extensive chemical simulant studies that were recently performed there. Also, the administrative and logistical aspects of the pilot study with a single state-level jurisdiction were anticipated to minimize coordination requirements that could negatively impact the test-pilot timeline. All of these criteria were drivers that ultimately contributed to the final decision of using the MBTA system as the location for the proposed pilot study.

Within the MBTA system, the three adjacent stations of Porter, Davis, and Harvard were selected as test locations due to previous airflow modeling efforts that could be leveraged for this test, as well as the ease of sensor installation in these locations. The tests are planned to begin in the spring of 2012 after the remodeling work is completed on these stations of the MBTA system.

3.1 Boston MBTA Subway Overview

The MBTA subway system is the nation's 5th largest mass transit system in terms of daily ridership. It serves a population of 4,667,555 in 175 cities and towns with an area of 3,244 square miles. To carry out its mission it maintains 183 bus routes, 2 of which are Bus Rapid Transit lines, 3 rapid transit lines, 5 streetcar (Central Subway/Green Line) routes, 4 trackless trolley lines and 13 commuter rail routes. The average weekday ridership for the entire system is approximately 1.1 million passenger trips, with 38% (heavy rail) of the 2009/2010 ridership on the red, orange and blue subway lines.[13,14]

The red line covers 21 miles of track, has 29 stations and offers 427 one-way weekday trips.[14] Operational hours for this line run from 5 AM until after midnight during weekdays and weekends. A trip between the stations of interest (Harvard Square and Davis Station) takes between 5 and 6 minutes. A commuter rail line which originates in Worcester County and travels through Middlesex County joins the red line at one of the test stations (Davis Station), bringing commuters from the suburbs of Middlesex County.

3.2 Air Quality

3.2.1 Boston Metropolitan Area

Air Quality in Eastern Massachusetts is not in attainment with the federal air quality standard for ground-level ozone, as mandated in the Clean Air Act. Massachusetts is in attainment for the other federal air quality standards to include carbon monoxide (CO), lead, nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and particulate matter (including PM10 and PM2.5). Ground-level ozone (O_3) is a colorless gas formed through a complex chemical reaction between volatile organic compounds (VOCs) and nitrogen oxides (NO_x) in the presence of sunlight. The state has developed and is implementing pollution control strategies to attain the ozone standard.[15]

3.2.2 Subway Indoor Air Quality

MBTA collected air quality data for carbon monoxide and respirable particulate matter over two weeks on indoor rail passenger platforms at the Back Bay commuter rail station. These commuter train platforms are serviced by diesel-powered locomotives. The monitoring was performed during the afternoon/evening peak service and the morning peak service on alternating days at two sites on the platform. Time-weighted averages over the 20-40 minute collection time for the respirable particulate matter for the locations sampled were 0.4 mg/m^3 each in the morning and 0.2 mg/m^3 each in the afternoon. For carbon monoxide levels, time-weighted averages over the 20-40 minute collection time at each of the two locations sampled in the mornings were 6 and 3 ppm, and 2 ppm each for the afternoon. The differences in morning versus evening concentrations are attributed to the increased number of inbound trains in the morning. Higher short-term measurements occurred throughout the study. This station has a co-located subway station allowing commuters to transfer between these methods of transportation. While the subway test locations use electric-powered trains, these data are presented as a reference point on indoor air quality during a MBTA commute.[16]

Particulate estimates from the Washington Metropolitan Area Transit Authority (WMATA) subway system operated in Washington, DC are also presented here as approximated values. The modern WMATA system-wide design to include air handling methods may exceed the air quality of the MBTA subway system which is more than 70 years older than the WMATA subway system. The data collected at WMATA would be considered a best case scenario, rather than a worst case scenario for indoor air quality, as compared to the MBTA.

WMATA conducted air sampling to collect information about worker exposure to particulate matter (bulk and airborne dusts) while performing tasks at different work site locations in the below ground tunnels of the five rail lines to provide an overview of the work environment. Both locational and personal air samplers

were used. Sample collection and analysis methods were carried out in accordance with National Institute for Occupational Safety and Health (NIOSH) protocols. Total dust sample results collected using personnel samplers on workers performing typical work tasks in the platform area of a Metrorail station ranged from 0.09-0.6 mg/m^3, with seven of the ten collected samples below 0.2 mg/m^3.[17] The samples collected identified that the majority of the dusts were smaller than 10 µm in size, and present below the Occupational Safety Health Administration (OSHA) permissible exposure limit (PEL) of 5 mg/m^3 for respirable nuisance dusts.[18] PEL is a time-weighted average over an 8-hour period. The dusts were considered to be "non-asbestos containing material."

Section 4. Environmental Consequences of Implementing the Alternative Actions

Information and evaluation data on the *B. subtilis* test material gathered from regulatory agencies and published documents are reviewed in this section to address the potential direct or indirect effects on health, safety, and the environment due to implementation of each alternative action. Portions of Alternative 2 and 3 will be discussed together as these alternatives vary only in the test conditions.

In addition to several published references cited below, the primary data sources for the human health and safety information used in the following evaluation regarding *B. subtilis* were:

1. The EPA review "*Bacillus subtilis* Final Registration Review Decision," dated 24 March 2010,[5] to support the registration and use of the *B. subtilis* biofungicides, and
2. An EPA Biotechnology Program Toxic Substance Control Act (TSCA) Final Risk Assessment for *B. subtilis*,[19] which evaluated *B. subtilis* bacteria used for the production of enzymes and specialty chemicals.

4.1 Human Health and Safety Effects

4.1.1 Test Material Exposure

The aerosol release of the particulate material into the subway station per Alternative 1 may result in rider contact with live *B. subtilis* spores, or with killed *B. subtilis* spores for Alternatives 2 and 3. Contact may include inhalation and/or ingestion of spores, as well as surface contact with exposed skin. Additional potential for contact may occur following testing due to the potential for re-aerosolation of the particulate material due to air movement within the subway station. Ridership during rush hours is expected to be largely comprised of healthy working adults, but young, aged or immune-deficient or immune-

compromised riders are also expected to be present during testing for Alternatives 1 and 2.

The Center of Disease Control and Prevention (CDC) and National Institutes of Health (NIH), Biosafety in Microbiological and Biomedical Laboratories,[11] defines four Biosafety Levels (BSLs), and recommends the BSL practices for numerous organisms. *B. subtillis* is determined to require BSL-1 practices, which is the least restrictive designation within this guidance. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended other than a sink for hand washing. BSL-1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans.

B. subtilis is approved for use as biofungicide,[5,8] human and animal food supplement,[7] and probiotic.[6,9] *B. subtilis* is ubiquitously found in soil and air and is generally considered to be an opportunistic organism with no pathogenic potential to humans.[5] The microbe is already a common element of food products consumed by the general public and used in food preparation,[27] and *B. subtilis* is not considered to be dangerous to consume.[6] Additionally, *B. subtilis* is sensitive to all antibiotics listed by the European Food Safety Authority,[6] indicating that the microbe is susceptible to all common treatments.

Table 1 below summarizes the findings of several government reviews and published literature on the safety and use of *B. subtilis* in agricultural and industrial settings. All of these studies were performed with living *B. subtilis* spores and cultures, as is proposed in Alternative 1. More detailed information, references, and analysis from each of these studies is provided in Section 7, but is summarized here for clarity.

For Alternatives 2 and 3, with killed material, the risk would be reduced even further as the material would have no opportunity to grow or infect, but would only be a concern as a nuisance dust. Table 2 below presents the comparison of particulate loads present in the system to the proposed test release.

Table 1. Summary of live *B. subtilis* safety studies and risk assessments relevant to Alternative 1.

Route of Exposure	**Doses Studied**	**Outcome**	**Calculated dose range for test***	**Risk assessment**
Inhalation[5,19]	1.1-3.4 x 10^8 cfu/rat	No adverse effects	3.5 x 10^5 - 1.88 x 10^6	Very Low – a rat is about 100

	1.5×10^6 cfu/person/year from release near industrial plant	No unreasonable risk	cfu/test	times smaller than a human
Ingestion[5,27]	1.1-3.4 x 10^8 cfu/rat	No adverse effects; all evidence of *B. subtilis* was cleared from internal organs within 14 days	No ingestion risk expected as a result of the test	None
	10^5 cfu/g was found in commercial food	No clinical studies were tracked, but *B. subtilis* is not a food disease agent even though it is present.		None
Skin contact[5]	3.6×10^9 cfu/guinea pig or rabbit for 24h	Temporary redness, swelling, and sores	No dermal contact expected as a result of the test	None
Eye contact[5]	1×10^9 cfu/rabbit	Irritation and conjunctivitis; cleared naturally in 4-7 days	3.5 x 10^5 - 1.88 x 10^6 cfu/test	Very Low

* Calculated dose of 10-50g releases, estimated assuming equal dispersion of particles over an entire station.

Table 2. Nuisance Dust Levels in Subways Relevant to Alternatives 2 and 3.

Route of Exposure	**PEL OSHA Maximum[18]**	**Typical Subway Air Quality**	**Calculated dose range for test***	**Risk assessment**
Inhalation[16,17]	5 mg/m^3 for 8-hour occupational exposure	0.2 – 0.6 mg/m^3 during rush hour	0.1 – 0.4 mg/m^3 for 2-hour duration	Very Low

* Calculated dose of 10-50g releases, estimated assuming equal dispersion of particles over an entire station.

The maximum total number of spores proposed to be released from a single test are 1.5×10^{12} cfu, however, since this amount will be rapidly distributed into an extremely large volume, a significant dilution effect will occur keeping exposure levels low. For Alternatives 1 and 2, a 10 foot radius controlled zone will be established on the platform around the release site with no access for the general public permitted while the release occurs. The material will be directed into the airspace to be rapidly mixed into the station, minimizing exposure of any one individual to *B. subtilis* spores. While the subway rider will not use personal protective equipment recommended for occupational exposure,[19] the challenge test material will be presented at or lower than the rates or concentrations assessed in the studies cited above. However, the precautions to be taken when *B. subtilis* is used in an agricultural or industrial setting take into consideration sensitization due to multiple exposures, and that an organism not ordinarily associated with disease processes in humans can act as opportunistic pathogens and may cause infection in vulnerable (young, aged, and immune-deficient or immune-compromised) individuals.[11] The proposed tests will only occur periodically throughout a one year timeframe, and below a frequency to expect sensitization from occupational exposure, therefore cumulative exposure effects are not expected due to these tests.

Data and resulting exposure risk assessments regarding the particulate material uses in agricultural and industrial manufacturing support the safety of the use of viable *B. subtilis* in validation testing of bio detection systems in the subway system. Tests for assessing human susceptibility were carried out at rates which allow for assessing risks due to occupational (applicator, manual harvest, industrial fermentation setting) exposures, and are considerably above the anticipated potential exposures due to testing in the subway. The high dose acute studies determined that the tested spores were not toxic, infective or pathogenic for the exposure routes of inhalation, ingestion and dermal contact. The use of killed *B. subtilis* would only further reduce any potential risk from this material in all of these same exposure routes.

4.1.2 Environmental Consequences for Alternative 1 on Human Health and Safety

The presence of riders from sensitive populations groups during testing presents additional health factors that must be considered for a safe and effective test for all subway patrons. While the probability that an infection of a vulnerable subway rider may occur is very low due to the small number of spores proposed to be released in the station during testing, the consequences of any infection caused

by the proposed testing are not acceptable and, as such, the use of viable spores in open air challenge testing of the biosensor system is not recommended.

4.1.3 Environmental Consequences for Alternative 2 and 3 on Human Health and Safety

Aerosol release of nonviable (killed) *B. subtilis* spores for testing sensors in the subway would eliminate concerns regarding the presence of immune-compromised or immune-suppressed riders during testing. The high energy rays used for irradiation damage and fragment the DNA of living organisms, inducing changes that make the organism unable to grow and return to a vegetative state.[20,21] This eliminates the potential for the spore to act as an opportunistic bacterium and be the causative agent of a bacterial infection. Therefore, the nonviable spore can be categorized as a nuisance dust when aerosolized, rather than a viable organism capable of growth.

Implementing Alterative 2 or 3 may affect overall testing of the detection equipment due to the potential for reduced assay sensitivity caused by irradiation of the test material. Differential effects for detection of viable versus nonviable materials have been observed based on the type of detection assay employed. Laboratory research assessing these effects can be conducted using the detection technologies to measure these potential effects, and ensure that these effects can be properly accounted for in the analysis of the senstivity limits of the equipment tested.[20,21]

Testing during non-revenue hours as described in Alternative 3 would greatly limit the exposure of subway riders to inhalation of the nonviable spore material because the aerosolized particles will have time to settle out of the air onto surfaces. Dermal exposure would still be possible, but a single patron would have to have direct contact with a large percentage of the subway station surfaces to accumulate a dose in excess of the dosage tested in the EPA study. The probability of this occurring is determined to be negligible. Testing during nonrevenue hours would eliminate the air movement effects caused by passengers in the stations, and this effect would have to be accounted for in the data analysis. However, trains can be run through the stations during the test, which is a dominant mechanism of particulate transport through the subway system.

Implementing either of these Alternatives with the use of the nonviable material will ensure that the health and safety of the subway riders, to include sensitive populations, will not be compromised as a result of the testing activities. These data further support the additional potential to test with the nonviable challenge material at higher quantities and concentrations than the viable material, or to conduct the releases of nonviable material when subway ridership is present in the station.

4.1.4 Environmental Consequences for Alternative 4 on Human Health and Safety

Release of *B. subtilis* spores directly into the sensors during operational hours for the subway eliminates the opportunity for direct contact of a subway rider (current or future) with the particulate material, and thus presents no potential for adverse human health or safety impacts. The test material would be directly captured by the biosensor. None of the test material would enter the station area. The material would be processed through and remain within the detection system waste collection containers. The direct challenge, however, eliminates the effects of air movement on the detection equipment, significantly reducing the capture of data necessary to fulfill the purpose of the test.

4.2 Indoor Air Quality

While direct data on the indoor air quality at the MBTA station platforms is not available, data from a commuter-rail train station operated by MBTA and subway trains operated in Washington, DC by WMATA indicate that the concentration of respirable particulates at these platform sites is a magnitude below the OSHA Permissible Exposure Level (PEL) and Particulates Not Otherwise Regulated (PNOR) limit of 5 mg/m^3 for respirable nuisance dusts.

4.2.1 Environmental Consequences for Alternatives 1, 2 and 3 on Indoor Air Quality

Implementation of Alternative 1, 2 or 3 would each release the same amount of particulate material of up to 50 grams per trial, no more than four times per month into the ambient air of a subway platform, for no more than 2,400 g total released over the course of the year. Seventy percent, or 35 grams, of the released challenge material is inert filler, which aides in dispersion, and is categorized as a respirable nuisance dust. The balance of the material is composed of the *B. subtilis* spores, also respirable in size. The spores would be killed in Alternatives 2 and 3, and would be considered a nuisance dust. Live *B. subtilis* spores used in Alternative 1 may not be considered nuisance dust, but would contribute the same amount of particulate matter into the ambient air on the subway platform.

It is estimated that the particulate material will remain airborne in the subway station for no more than 2 hours following the release. The calculated air concentrations during this 2-hour timeframe remain well below the PEL level of 5 mg/m^3 for respirable nuisance dusts and PNOR limits.[18] The PEL is calculated over an 8 hour time-weighted average, while the particulate material test release is not expected to exceed 2 hours of airborne time. This shorter-term addition of

particulate to the indoor air quality further reduces the air concentration when compared to calculations over an 8 hour time-weighted average.

Implementation of any of the alternatives in combination with potential background dust in the MBTA is not expected to degrade the indoor air quality using the PEL for respirable nuisance dust in the workplace as a reference for indoor air quality. Due to the low frequency of test events, normal air exchange between the stations and the outdoor environment are expected to return all particulate levels to normal.

4.2.2 Environmental Consequences for Alternative 4 and the No Action Alternative on Indoor Air Quality

Implementation of Alternative 4 or the No Action Alternative would not release any quantity of respirable particles into the ambient air of the subway station, and thus would have no potential to impact indoor air quality at the subway.

4.3 Environmental Effects on Wildlife

The potential for exposure of terrestrial wildlife to the *B. subtilis* spores due to movement of the material with the air vented from the station, or with the train as it travels out of the station and above ground was evaluated. Due to the ubiquitous nature of *B. subtilis* in the environment, and the low to no toxicity of the organism, this effect will not impact the surrounding environment.

Exposure studies conducted to determine the effects to terrestrial wildlife during the application of *B. subtilis* as a biofungicide resulted in a determination that no unreasonable adverse effects to non-target organisms or the environment are likely to result from use of the *B. subtilis* products according to the label.[19]

Oral dosing with *B. subtilis* alone at up to 3,000 mg of product /kg of body weight, or about 600 mg/bird was not infective or pathogenic to Bobwhite quail as determined by necropsy.[5] No treatment-related effects on weight gain or feeding consumption occurred through the study. Feeding studies with some invertebrates did produce mortality when fed at 10 to 100 times the expected environmental concentration due to application of the *B. subtilis* biofungicide.[5] Sprays of the biofungicide had no effect on the emergence of larvae of the beneficial insects tested.[5] Acute toxicity studies conducted on rodents for use as models in determining human health effects concluded that *B. subtilis* was not toxic, infective or pathogenic for rats when dosed orally and intratracheally.[5]

Viable *B. subtilis* spores administered at doses greater than the proposed test concentrations in the subway have been studied and were determined not to have an adverse effect on the terrestrial wildlife.[5] Therefore, the release of the entire test volume of viable spores will not impact terrestrial wildlife which may

become exposed to spores leaving the subway station. The use of the nonviable spores will also have no adverse effect on terrestrial wildlife as these spores, if ingested or inhaled will act as a nuisance dust incapable of infecting the contacted animal.

4.4 Environmental Compliance

Equipment used to generate the aerosol release of the particulate material will be returned to the laboratory, cleaned and evaluated for reuse. Solid and liquid waste generated by the biosensor systems during the automated sample analysis and decontamination/cleaning steps will be collected and contained within the sensor system. The waste reservoir will be manually removed from the system and disposed as solid waste. None of the analytical reagents or cleaning solutions is Resource Conservation and Recovery Act (RCRA) regulated hazardous waste. The wastes generated will not significantly differ between the any of the Alternatives as they involve the challenge of the detection system across the subway.

4.5 Environmental Justice

In accordance with Executive Order 12898,[22] analysis of the environmental effects must include effects on minority communities and low-income communities, when such analysis is required by the National Environmental Policy Act of 1969, 42 U.S.C. section 4321 et esq.

The populations of the cities of Cambridge and Somerville which surround the MBTA stations of interest, and the county of Middlesex, which envelops these stations and a connecting commuter rail service, have minority and low-income populations, as well as moderate and upper income populations. For this analysis ridership at these stations is projected to be residents of the cities local to the stations, and residents from Middlesex County serviced by the commuter rail which services Porter Station.

In 2008, 8.1% of persons in the county of Middlesex were living below the federal poverty threshold, as compared to 10.1% of the population of the state of Massachusetts. Minorities in Middlesex County account for 20% of the population, which mirrors the 21% minority population for the state of Massachusetts. Median household income in Middlesex County in 2008 exceeded the State average by 16%.[23]

Population data on poverty thresholds for the cities of Cambridge and Somerville are available for the year 1999, and reflect that 3.5% more of each of these the city's residents were living below the federal poverty level as compared to the State as a whole during that time frame. Minority populations accounted for 16%

more of Cambridge residents and 7% more of Somerville residents than the State as a whole. The city of Cambridge population trends, when compared to the State of Massachusetts indicate representation of a student population. Cambridge has two times the number of persons with Bachelor's degree or higher (65% Cambridge vs. 33% MA), two times the number of foreign born persons (26% Cambridge vs. 12.2% MA), and half the number persons under the age of 18 (13.3% Cambridge vs. 23.6% MA).

Implementation of the presented alternatives has no adverse impact on resources, human health or the environment. No mitigation measures have been identified as a requirement to carry out any of the possible alternatives because there is no evidence that any low income or minority populations would receive a higher exposure to the particulate matter than any other group. Therefore, selection of any of these alternatives would not disproportional impact minority or low-income communities.

4.6 Historic Properties

Pursuant to Section 106 of the National Historic Preservation Act of 1966 (NHPA) and its implementing regulations at 36 C.F.R. Part 800, consideration was given to the impact of the tests on any historic properties. Harvard Station, the oldest stop of the test locations, opened in 1912. However, major construction was done to the station and tracks in 1981, and the original station was decommissioned and closed down when the new Harvard Station opened. The other two stations, Porter and Davis, both opened in December 1984 as part of the Red Line Northwest Expansion project. All of the stations in which the proposed action will take place are less than 50 years old, and none are listed on the National Register of Historic Places.[24]

A letter was sent to the Massachusetts Historical Commission (MHC) on January 11, 2012 describing the proposed action and the finding that no historic properties would be affected by the placement of sensors in the MBTA stations.

Section 5. Conclusions and Identification of the Proposed Action

The proposed *B. subtilis* test material is a commercial product which has undergone rigorous studies to evaluate the potential health effects and safety of the material for the general public, workers and environments surrounding the commercial use. No adverse health effects from low level exposure to *B. subtilis* in healthy populations have been documented. Scientific sources suggest that strains of *B. subtilis* spores registered by the US EPA for use as a biofungicide continue to meet the statutory stand of no unreasonable adverse effects to

human health, including occupational and non-occupational exposures, or the environment including environmental fate and nontarget organism. The EPA registration review decision for these products determined "…that there are not like to be any unreasonable adverse effects to the U.S general population, and to infants and children in particular, or to non-target organism or the environment from the use of registered pesticide products containing *Bacillus subtilis* when currently required labeled instructions are followed."[5]

The test challenge quantities being used are equivalent to or below the dose rates for the toxicology testing of these biofungicides, and the reported results provide a conservative comparison. The risk assessments regarding human health and environmental effects to target and non-target organisms can be applied to environmental assessment of the effects of these products used in accordance with the proposed action, and across the alternatives which release the *B. subtilis* spore. These data provide a reasonable expectation that the use of this product as outlined in the proposed action will not result in any environmental impacts, and that the health and safety of the healthy population of subway riders will not be compromised. However, precautions due to the use of the subway by riders from sensitive populations such as the immune-compromised must be considered. The potential for the otherwise non-pathogenic *B. subtilis* to act as an opportunistic pathogen and cause infection in vulnerable individuals must be considered in the decision making process.

Due to the health-related concerns over the potential presence of a small minority of the general public who may be immune-compromised, the use of viable organisms will not be pursued in the proposed challenge tests. However, considering the data collected in the numerous studies cited throughout this assessment, as well as the low doses that are proposed for use in the study and the natural exposure already experienced by the public through the environment and food supply, the use of the killed material described in Alternatives 2 and 3 is considered safe for the public. The use of the killed material eliminates the potential for the spore to germinate and grow and avoids the possibility of producing infection in riders, but still presents a material to the sensors which will test their detection performance and capability.

The difference between Alternate 2 and 3 is in executing the tests during either revenue or non-revenue hours. While the movement of people within the subway system is expected to help move the particulate material, the movement of trains between the stations is a very strong effect in pushing the material between stations. During Alternative 3, trains will be run between the stations for the test events to mimic this transport mechanism and so will provide a realistic estimate of the motion of material within the stations. Thus, Alternative 3 will not substantively change the performance evaluation but will reduce the risk of high dermal or inhalation exposure of the public to the particulate material.

Considering all of the concerns and studies examined above, Alternate 3 is the Proposed Action for these tests. This Alternative is considered to be the best balance of the risk of exposure to the public while maintaining the benefit for understanding the performance of these vital safety systems. DHS S&T provided two opportunities for the public to comment or raise concerns about the safety and analysis of this Proposed Action. This document was posted on a website with an email address open for comment during a 6-week period from April 30 to June 15, 2012. Also, a public forum was held in Cambridge, MA on May 16, 2012. Public input submitted through these methods did not result in any changes to the conclusions in the EA or the Proposed Action. Therefore, the Proposed Action will be pursued to conduct the tests described above.

Section 6. Persons and Agencies Contacted

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Section 7. Additional Information and Analysis of *B. subtilis*

Documented safety studies for *Bacillus subtilis* as a fungicide:
As previously mentioned, four strains of *B. subtilis* spores have been registered by the US EPA for use as a biofungicide on food crops,[5] examples of which include leafy vegetables, stone and pome fruits, tomatoes, walnuts, cucurbits, peppers, and turf. The biofungicide formulations may be applied to the crops as often as every seven days and the food crop may be harvested on the day of treatment. Per the requirements of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)[25], the EPA evaluated the *B. subtilis* biofungicides for potential direct and indirect human and environmental hazards prior to registering the products for sale and use on food crops and plants. The evaluations require a series of standardized product quality and toxicity tests on which to base an assessment of the risk to workers and the general population (human risk assessment), as well as to predict the environmental fate and effects on target and non-target plants and animals (ecological risk assessment).

In March 2010, the EPA completed a final FIFRA registration review decision for the *B. subtilis* products. The review was part of a periodic reevaluation of registered products to make sure that as the ability to assess risk evolves and as science, policies and practices change, all registered products continue to meet the statutory standard of no unreasonable adverse effects to human health, including occupational and non-occupational exposures, or the environment.[5] The final review followed a Final Work Plan that addresses public comments received concerning information provided on what the EPA knows about the materials, and what additional test data and analyses the EPA required to make the decision on the registration review. The EPA registration review decision for these products determined "…that there are not likely to be any unreasonable adverse effects to the US general population, and to infants and children in particular, or to non-target organism or the environment from the use of registered pesticide products containing *Bacillus subtilis* when currently required labeled instructions are followed."[5]

The EPA Final Registration Review Decision for the biofungicides determined that dietery exposure risks to adults, infants and children were miminal due to the low acute oral toxicity/pathogenicty potential for the *B. subtilis* strains. The review for dietery risk exposures concluded "…there is a reasonable certainity that no harm will result from aggregate exposure to the US population, including infants and children, to the residues of these strains of *B. subtilis*. This includes all anticipated dietary exposures and all other exposures for which there is reliable information."[5] The risk assessment also determined that occupational exposure to *B. subtilis* is not expected to pose undue risk as acute toxicity and pathogencity studies have not shown any toxic or pathogenic effects to rats via oral, pulmonary, dermal and intravenous routes of exposure. Regardless, personal protective equipment for the applicator and delayed worker re-entry

intervals for crops treated with *B. subtilis* are required to mitigate any potential dermal or inhalation risks due to prolonged exposure.[5]

Several commercial fungicide products containing *B. subtilis* also bear the seal of the Organic Materials Review Institute (OMRI),[26] an organization that provides organic certifiers, growers, manufacturers, and suppliers with an independent review of products intended for use in certified organic production, handling, and processing. Based on OMRI review findings and US Department of Agriculture (USDA) requirements, food treated with these *B. subtilis* biofungicides maintain the USDA organic standing. By the nature of its application in commercial agriculture, human populations have been exposed to the *B. subtilis* challenge test material with no documented adverse reaction.

Based on the surface area of the subway station test zones, the maximum test challenge release of 50 g is the same area concentration as one of the lower labeled EPA approved use rates for application to a food crop for a commercial biofungicide formulation with a similar concentration of *B. subtilis*. This assumes that all of the released material would be contained within the single station. All airflow modeling[4] and initial test results show that particles are transported quickly to other stations within the system, and thus the deposited concentration within the release station will be much lower than this estimate, and therefore much lower than the EPA approved concentration.

Reviews of commercial manufacturing process, formation of unintentional ingredients, stability and sample analysis for the *B. subtilis* biofungicide products were found acceptable in meeting the EPA product standards.[5] The quality controls and good manufacturing practices implemented by the manufacturers and enforced by the EPA regulation of these products assures minimal to no variablity in the test material. Thus, selection of a well characterized commercial *B. subtilis* product for use in testing under the proposed action will assure health and safety aspects associated with the laboratory manipulation and test release of the material are understood. The assurance that unintentional ingredients or potential impurities will not be found in the test challenge material eliminates the potential for unforeseen adverse health impacts during testing due to these contaminants. In addition, the use of a well characterized challenge test material assures the quality and consistency of the data collected over the course of the test.

<u>Documented safety studies for *Bacillus subtilis* as a food supplement</u>:
In addition to its use as a fungicide, *B. subtilis* is also used as a human and animal food supplement and probiotic. A study assessing chronic toxicity in mice, rabbits and pigs showed no signs of toxicity or histological changes in organs or tissue, and concluded that the data support the use of *B. subtilis* as a food supplement and may be considered as non-pathogenic and safe for human consumption.[6] Additionally, *B. subtilis* was sensitive to all antibiotics listed by the

European Food Safety Authority, indicating that any potential ensuing infection caused by the microbe is easily treatable.

To further highlight the prevalence of *B. subtilis* in our food products, a 1996 study sampled purchased food from retail stores in the Netherlands for the presence of live *B. subtilis* and its close relative *Bacillus cereus*. Milk samples represented 68% of the total number of test samples, with the remaining 33% of samples being collected from meat, pasta, spices, bakery products, cocoa and Chinese meals. None of the 157 milk samples were positive for *B. subtilis*, while 36% of these samples were positive for *B. cereus*. Each of the six spice samples collected tested positive for both *Bacillus* species through culture analysis, ranging from 10×10^2 to 10^5 cfu/g. The remaining 45 samples of meat, pasta, bakery products, cocoa and Chinese meals had between 10 and 25% of the samples test positive for *B. subtilis*.[27]

These studies show that *B. subtilis* is already a common element of food products consumed by the general public and used in food preparation, and that *B. subtilis* is not considered to be dangerous to consume, as many of the food products found to contain this material do not have any associated food handling instructions to minimize exposure of the general public to this organism.

<u>Documented safety studies on other routes of *Bacillus subtilis* exposure</u>:
The EPA assessment under TSCA reviewed potential *B. subtilis* inhalational exposure resulting from routine releases at large scale, conventional fermentation facilities. The data were obtained from eight pre-manufacture notices submitted to EPA under TSCA, and from information collected on the fermentation of non-engineered microorganisms by a NIOSH walk-through survey of several fermentation facilities in the enzyme industry. Inhalation exposures due to air releases from a fermentor off gassing into the air outside of the facility via air vents estimated a potential human inhalation dose rate ranging from 3.0×10^3 to 1.5×10^6 cfu/year.[19]

The TSCA review used these data to conclude that "The use of *B. subtilis* in an industrial setting should not pose an unreasonable risk to human health or the environment. First, human health and environmental hazards of *B. subtilis* are low. Second, the number of microorganisms released from the fermentation facility is low."[19] The review concluded that although not completely innocuous, the industrial use of *B. subtilis* presents low risk of adverse effects to human health or the environment.[19]

An estimated inhalation dose for a subway rider during a single release test during rush hour with a wait time of 10 minutes on the platform would be on the order of 1.8×10^6 cfu. This estimate assumes that all released material is contained within the single station, which is a worst-case scenario estimate as most models[4] show airflow between stations occurs quickly, which would rapidly decrease the exposure of any one person that may be present during a test

release. This dose is comparable to the calculated inhalation exposures of up to 1.5×10^6 cfu for persons breathing air vented to the outside environment from a fermentation facility.

The TSCA evaluation for the use of genetically modified *B. subtilis* reviewed the opportunistic pathogenicity of the unmodified *B. subtilis*. The document stated that "reviews of *Bacillus* infections from several major hospitals suggest that *B. subtilis* is an organism with low virulence."[19] In another hospital study over a 6-year period, only two of the 24 cases of bacteremia caused by *Bacillus* (of a total of 1,038 cases) were due to *B. subtilis*. Many of these patients were immuno-compromised or had long term in-dwelling foreign bodies, such as a Hickman catheter.[19] A 1991 review reported that most cases of human infection are due to the parenteral introduction of spores either in IV drug users on dirty needles or through trauma.[28] *B. subtilis* is ubiquitously found in soil and air and is generally considered to be an opportunistic organism with no pathogenic potential to humans.[5] As further evidence of its ubiquitous presence in the environment, a 1978 survey of bacterial flora in 21 homes found *Bacillus* species in kitchens at 17 of the sites and in bathrooms at 16 sites.[28] Since *B. subtilis* is virtually everywhere it is inevitable that it may be found in association with other microbes in infected humans, however, according to Edberg, 1991, either the number of microorganisms challenging the individual must be very high or the immune status of the individual very low in order for infection with *B. subtilis* to occur.[19]

The following sections summarize additional animal studies on exposure routes for *B. subtilis* used in the EPA registration review.[5] No studies or results were found in the published literature in contradiction to the conclusions presented here.

<u>Inhalation and Ingestion</u>: Acute toxicity studies were conducted via oral and intratracheal (pulmonary via percutaneous injection into the trachea for the delivery into the lungs) routes in male and female rats to support the use of *B. subtilis* as a biofungicide.[5] Each study concluded that the *B. subtilis* was not toxic, infectious or pathogenic for rats when dosed orally and intratracheally. The dose per rat ranged from 1.1×10^8 to 3.4×10^8 cfu/animal.[5] Results of the oral dosing studies showed no adverse clinical signs and gross necropsy did not reveal any abnormatilies. *B. subtilis* organisms were found to clear from all tissues beween 14- and 21-days post exposure. Acute pulmonary studies observered no adverse effects in the gross necropsy, however the *B. subtilis* continued to be detectable in the lungs at 35 days post dose.[5] Given that the average mass of a male Sprague-Dawley rat is ~0.5 kg and the average mass of an adult male is ~80 kg, the acute exposure that could result from any of the proposed alternatives through inhalation or ingestion is, on a per mass basis, orders of magnitude lower than the levels cited in the above studies.

Dermal Contact: Manufacturers of *B. subtilis* biofungicides have reported no personnel incidents of allergic reactions resulting from repeated expsoure (hypersenitivity) to *B. subtilis* during testing, production, or use of the *B. subtilis* products. Moderate skin sensitization reactions that include redness, swelling, and sores were observed when *B. subtilis* at concentrations up to 3.6×10^9 cfu was applied to the skin of clipped rabbits and guinea pigs for a 24-hr exposure period.[5] Again, these exposure doses are significantly higher than any exposure that would be anticipated from the proposed tests. The maximum total number of spores proposed to be released from a single test are 1.5×10^{12} cfu, however, since this amount will be rapidly distributed into an extremely large volume, a significant dilution effect will occur keeping exposure levels low. Care will be taken in the execution of the tests to ensure that the material is not directed at exposed skin on a single person, but that the material is directed into the airspace to be rapidly mixed into the station. For the releases, a 10 foot radius controlled zone will be established on the platform around the release site with no access for the general public permitted while the release occurs.

Acute Eye Irritation: Rabbits dosed with 0.1 g (equivalent to 1×10^9 cfu) in their eyes exhibited slight to severe irritation and conjunctival effects which cleared from 4–7 days post dosing.[5] Care must be taken in the execution of the test release to ensure that the material is not directed at any person's eyes to mitigate this risk. However, even with large exposure doses in the eyes, no permanent health effects were noted in the study.

Section 8. References

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