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Review of Decontamination Techniques for the Inactivation of Bacillus anthracis and Other Spore-Forming Bacteria Associated with Building or Outdoor Materials

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Abstract

Since the intentional release of Bacillus anthracis spores through the U.S. Postal Service in the fall of 2001, research and development related to decontamination for this biological agent have increased substantially. This review synthesizes the advances made relative to *B. anthracis* spore decontamination science and technology since approximately 2002, referencing the open scientific literature and publicly available, well-documented scientific reports. In the process of conducting this review, scientific knowledge gaps have also been identified. This review focuses primarily on techniques that are commercially available and that could potentially be used in the large-scale decontamination of buildings and other structures, as well as outdoor environments. Since 2002, the body of scientific data related to decontamination and microbial sterilization has grown substantially, especially in terms of quantifying decontamination effcacy as a function of several factors. Specifically, progress has been made in understanding how decontaminant chemistry, the materials the microorganisms are associated with, environmental factors, and microbiological methods quantitatively impact spore inactivation. While advancement has been made in the past 15 years to further the state of the science in the inactivation of bacterial spores in a decontamination scenario, further research is warranted to close the scientific gaps that remain.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b05274.
Synopsis and table of emerging decontamination techniques; techniques for the inactivation of *C. difficile* spores; review of other sporicidal techniques such as calcium hypochlorite, ozonated water, and glutaraldehyde (PDF)

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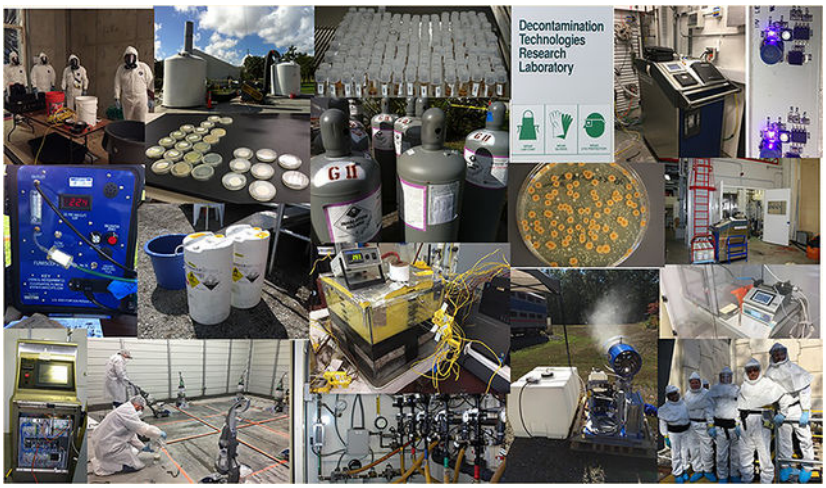

INTRODUCTION

Although the military has been developing decontamination methods for their purposes for decades,[1] research conducted or funded by nondefense government agencies with a focus on the decontamination of civilian facilities had been minimal prior to 2001. That changed following the intentional release of *Bacillus anthracis* spores through the U.S. Postal Service in the fall of 2001[2] (referred to as the Amerithrax attack), with research and development (R&D) related to decontamination for this bioterrorism agent increasing significantly since then.[3] (*B. anthracis* is the bacterium causing anthrax disease, and can infect livestock, wildlife, and humans.[4]) One driver for this new research was the overall cost of the remediation efforts across the United States, which was estimated to have been approximately $320 million.[5] It required the use of sporicidal chemicals to essentially sterilize large buildings, which was unprecedented.[6] This is because bacterial spores are one of the most resistant microbial forms to inactivate with biocides,[7] and may survive for centuries if left undisturbed.[8] When aerosolized, bacterial spores such as those of *B. anthracis* can remain aloft for hours and thus have the potential to widely disperse,[9] greatly expanding the extent of contamination, and further exacerbating recovery efforts.

Other more recent, unintentional incidents of *B. anthracis* contamination, whether naturally occurring[10] or manmade,[11] continue to demonstrate the need to advance decontamination science and technology. Examples include several incidents of contamination and numerous fatalities resulting from drumming-associated activities or heroin use, both traced to *B. anthracis*-contaminated goat skins.[12] The recently developed National Biodefense Strategy further attests to the need to develop decontamination approaches for all biological threats, regard-less of their origin.[13]

A few years following the Amerithrax incident, a review of *B. anthracis* spore inactivation techniques[2] and a compilation of building decontamination alternatives[14] were published. Two other articles have been published since then that provide overviews of decontamination approaches for *B. anthracis*.[3,15] While these previous review articles provide helpful, qualitative summaries of decontamination approaches and operational aspects that could be

employed after a release of *B. anthracis* spores, they generally lack detailed data to document the conditions in which the decontaminants are effective.

This current review highlights the scientific and technological advances made relative to *B. anthracis* spore decontamination technologies since these initial reviews, that is, from approximately 2002, and identifies knowledge gaps. The scientific advances gained over the past 15 years or so primarily include the development of a large amount of data and information related to the chemistry and environmental conditions in which these decontaminants are effective. Thus, we have taken a more quantitative approach, vis-à-vis the synopsis of effcacy data for numerous decontamination techniques. The data are presented as a function of the materials the spores are deposited on, and also other important factors such as the chemistry (e.g., active ingredient concentration, contact time, dosage) and environ-mental (e.g., materials, temperature, humidity) conditions in which they are effective. Lastly, while the primary focus of our review is on *B. anthracis*, we present data for other spore-forming microorganisms as well. Many of the techniques discussed in this review for the inactivation of *B. anthracis* spores can also be effectively used for other virulent spore-producing bacteria that present public health concerns.

MATERIALS AND METHODS

There are numerous bacterial spore inactivation techniques of various scale, readiness, and application. Therefore, to provide a more succinct review, we have limited the scope to techniques that can be used at a relatively larger scale (such as for a building) and are commercially viable. Further, our review focuses on both liquid- and gaseous-based chemistries, as well as a few physical-based techniques. Liquid decontaminants are primarily used for surfaces, whereas gases are employed for volumetric decontamination, that is, inactivation of spores on surfaces as well as aerosolized spores, in large enclosed areas. Additionally, the following is a review of the scientific literature, limited to peer-reviewed journals and government reports, if the reports are publicly available and the methods are suffciently documented.

Scope of Technologies Under Review.

While there are well-established techniques for sterilization of materials such as foodstuffs, medical instruments, and pharmaceuticals, these techniques are typically confined for use on a small scale or may not be easily transportable (e.g., because of the use of hazardous or radioactive materials). Examples include ethylene oxide (which is flammable and carcinogenic),[16,17] as well as ionizing radiation in the forms of gamma irradiation,[18,19] X-rays,[20] and electron beam.[21] Although excluded from the scope of this review paper, some of the above techniques were used to decontaminate certain items following the Amerithrax attacks,[3] and thus would be considered as decontamination tools for small, valuable, or personal items that would be sensitive to chemical exposure (e.g., cash, mail, jewelry, artwork, mechanical devices, electronics).

There are several emerging decontamination and sterilization techniques undergoing extensive R&D, such as the use of cold atmospheric plasma.[22,23] For these newer techniques that have undergone significant development but are not quite commercialized, we have

provided a brief synopsis in the Supporting Information (SI). While physical removal of bacterial spores from a substrate (e.g., via washing or vacuuming[24,25]) may be considered "decontamination", and may play a role in an overall remediation plan for a wide area contamination event,[3] for brevity we have focused this review only on techniques that inactivate spores (e.g., chemical, irradiative). Prophylactic approaches that would inactivate spores that come in contact with a material embedded with an antimicrobial[26,27] are also excluded from this review.

Since the focus of this review is on decontamination, which implies the presence of a surface, material, fomite, or reusable object, our review generally excludes spore inactivation techniques or studies that do not involve the use of substrates, such as those involving liquid suspensions, water treatment,[28] water infrastructure,[29] and wastewater treatment.[30] Spores are much more difficult to inactivate when they are deposited on a surface compared to being suspended in a liquid.[31] Similarly, spores suspended in air are more readily inactivated (at least by gaseous or physical-based decontaminants) compared to spores associated with a surface, since there are no chemical and/or physical interactions between the microorganism, the material, and the decontaminant to diminish effcacy. For this reason, we have this excluded aerosol studies (e.g., see Grinshpun et al.[32]). Nevertheless, we acknowledge that the health effects associated with inhalation of aerosolized *B. anthracis* spores are more severe (higher morbidity and mortality) than with dermal contact of a contaminated surface.[33]

As we emphasize throughout this review, the contaminated material is a predominant factor in the effcacy of the technology and has often been overlooked in previous reviews for spore inactivation or decontamination techniques. For example, hard, nonporous, and inorganic materials (e.g., glass, stainless steel) are typically easier to effectively decontaminate than porous or organic materials (e.g., ceiling tile, soil). From a mechanistic standpoint, many decontaminants rely on oxidation chemistry, and so spores associated with organic materials tend to be inactivated less effectively. Porous materials may provide micro-locations for spores to escape contact with decontaminants. These material interactions and chemical mechanisms affecting effcacy are discussed throughout this review. Thus, we synthesize in this review the chemistry of the decontaminants as well as the complexity of surfaces and materials found outside a hospital, clean room, or laboratory that make the task of decontamination following a *B. anthracis* spore release highly challenging, and for which rigorous effcacy testing using realistic materials and applications is critical.[34]

Focus on Decontamination Effcacy.

We emphasize in this review decontamination effcacy, which is typically reported in terms of $\log_{10}$ reduction (LR), in which LR = $\log_{10}$ (mean CFU (colony forming units) recovered from positive control carriers) − $\log_{10}$ (mean CFU recovered from decontaminated carriers).[35] (Positive controls are the carriers or coupons inoculated with bacterial spores but not exposed to the decontaminant.) Effcacy is a function of the myriad experimental variables that may be investigated, and as discussed above, the contaminated material is a major factor. (The presence of soil or organic loading on a material may also diminish the decontaminant's effcacy, especially if the spore inactivation mechanism for the

decontaminant is oxidation. Therefore, most registered antimicrobials require a clean surface before use.) Other factors affecting effcacy that we discuss in this review include decontaminant chemistry and related characteristics (e.g., chemical concentration, mass applied to surface, contact time (CT)), environmental conditions (e.g., temperature, relative humidity (RH)), and microbiological factors (species, strain, spore loading,[36] spore preparation, inoculation method,[37] extraction method, active ingredient neutralization method, etc.). This multitude of factors that may affect effcacy has typically been poorly elucidated in previous reviews. Most of the techniques we review here utilize chemical-based sporicides, but we also discuss two physical-based sterilization techniques (e.g., heat treatment[38] and ultraviolet light[39]). Nearly all the tests described in this review were conducted at laboratory ambient temperature (20−25 °C); decontamination effcacy data for lower temperatures (pertinent to outdoor decontamination) is a research gap.

In this review, we refer to a decontamination technique as “effective” against bacterial spores on a material if effcacy is ≥6 LR. Note, however, that a decontamination test result may also be considered effective if the population of spores on the material is completely inactivated, even when the recovery of spores from the positive control is less than 6 log CFU and hence resulting in <6 LR. The 6 LR criterion originates from guidance established for effcacy testing of antimicrobial products with claims to inactivate *B. anthracis* spores on inanimate surfaces,[40] using sporicidal test methods such as AOAC International Method 2008.05[41] with virulent *B. anthracis* spores or a surrogate. Additionally, refer to Ryan et al.[37] and other references[7,42] for further information related to the use of effcacy test methods for antimicrobial pesticides and sporicidal decontaminants, how test methods and carrier material can affect effcacy results, and related policy implications thereof. Larger-scale decontamination tests utilizing an aerosol release of spores may use surface sampling followed by analysis using culture or PCR to characterize effcacy.[43]

To allow for reporting additional granularity of data, in this review we refer to a decontamination test result as “moderately effective” if LR is 3.00−5.99, and “ineffective” if LR is <3.00. We acknowledge that some sporicidal effcacy test methods and associated policy result in only a “pass/fail” determination, and do not allow for use of a “moderately effective” nomenclature. Lastly, in an actual contamination incident with *B. anthracis* spores, offcials may require no detectable spores as a remediation goal, and thus the required LR in an actual incident would depend on the contamination level. Alternatively, at some point it may become too expensive or disruptive to require decontamination in a building with very low, but detectable, levels of *B. anthracis* spores.[44]

Although we focus primarily on decontamination effcacy in this review, there are other criteria for selecting a decontamination method. These may include whether the technology has been demonstrated at full-scale, its cost, availability (technology, chemicals, expertise, personnel), material compatibility,[45] health and safety (most techniques are hazardous) issues, and environmental impacts.

B. anthracis Strains and Other Spore-Forming Bacteria Included in Review.

While the primary focus of our review is for *B. anthracis*, we have also included data from other Bacillus species. Additionally, most of the decontamination data in the literature for

spores of virulent *B. anthracis* are from tests using the Ames strain. Very few effcacy data for other virulent strains such as Vollum[31] are reported (this is a gap). As such, unless otherwise noted, "*B. anthracis*" in this review refers to the Ames strain. Occasionally, the avirulent vaccine strain "Sterne" is used in tests.

This review also includes decontamination effcacy data for other spore-producing Bacillus species, which may be tested as surrogates for *B. anthracis* or other microbial contaminants. While Bacillus atrophaeus has long been used by the biodefense community as a simulant,[46] we have included surrogate data only if previous experimentation included both *B. anthracis* and the surrogate organism, and that the surrogate demonstrated similar or greater resistance to inactivation compared to *B. anthracis*. The data we have included in this review do confirm the historical use of B. atrophaeus (aka Bacillus globigii aka BG) and its phenotypic relative Bacillus subtilis as appropriate surrogates for *B. anthracis* when decontaminating with chlorine dioxide gas,[47] hydrogen peroxide vapor,[48] formaldehyde gas,[49] ozone gas,[50] and several liquid sporicides such as peracetic acid (PAA), aqueous hydrogen peroxide, hypochlorite, and aqueous chlorine dioxide.[31,51,52] In the following sections, other Bacillus data may also be presented for a decontaminant according to the criteria noted above. Interestingly, whereas *B. anthracis*, *Bacillus thuringiensis*, and *Bacillus cereus* may be considered the same species based on genetic evidence,[53] *B. thuringiensis* or *B. cereus* have not often been used[31] in decontamination studies, although their use is gaining traction; see for example Sagripanti et al.[31] and Buhr et al.[54–56]

This review of bacterial spore inactivation techniques may be applicable for other virulent spore-producing bacteria, such as *B. cereus, Clostridium botulinum*, and *Clostridium difficile*. Because C. difficile is a major public health concern and a source of nosocomial infections,[57] we have presented further discussion of inactivation techniques and data for this spore-former in the SI.

Physiology of Bacterial Spores Relative to Inactiva-tion Mechanisms.

While the primary focus of this review is to elucidate the chemical or physical treatment conditions con-ducive to effective inactivation of bacterial spores as a function of substrate (i.e., at the macro scale), we also briefly present here and throughout the review the microbiological mechanisms thought to be responsible for inactivation of bacterial spores. Some good overviews related to spore physiology and sporicidal mechanisms may be found elsewhere.[7,8,58,59] Briefly, spore killing mechanisms may involve damage to one or more of the following cellular components or metabolic activities, and depend on the decontaminant chemistry: damage to DNA, the inner membrane, the germination apparatus, or inactivation of core enzymes. The structure of bacterial spores, including the presence of an intact spore coat, plays a major role in their resistance to inactivation.[59]

LIQUID-BASED SPORICIDES

The application of liquid-based decontaminants is a simpler approach than the use of gaseous decontaminants (here occasionally referred to as fumigants) and may be more amenable for use by a facility owner/occupant in the event of an intentional wide-area *B. anthracis* spore release.[60] In addition to chemical decontaminant parameters such as active

ingredient concentration and CT that affect effcacy, the pH of the liquid and the mass of active ingredient applied to a surface are other operational parameters that may affect effcacy and are reported here if available and relevant.

One advantage of using liquid decontaminants is that they may be applied to surfaces or materials using a variety of different techniques. These application methods include spraying (usually low pressure is preferred to minimize reaerosolization of spores), immersion (may be useful for materials that would be discarded as waste), fogging (generation of microscopic droplets; useful for volumetric or surfaces), gels (use of a binding agent such as fumed silica; may be useful for vertical surfaces or ceilings), foams (entrained air; vertical surfaces, ceilings), prewetted wipes (useful for small items with complex surfaces), and simply using a mop or sponge. With regard to spraying or fogging, an electrostatic charge may be applied to the droplets generated to improve their adherence to complex surfaces (as is done routinely in other applications, such as in agriculture[61]), although the documentation of improvement in effcacy is lacking. In addition, although foams and gels may allow for longer CT of the liquid decontaminant on a surface due to their ability to adhere (important for a ceiling or a vertical surface), the mass of active ingredient in actual contact with the surface may be less due to the thickness of the foam or gel; research to clarify whether foam or gelling agents improve decontamination effcacy of liquid sporicides is also needed. One drawback with the use of liquid sporicidal chemicals is that they're primarily used for surface decontamination (with fogging an exception) and not for killing aerosolized spores. To what extent surface decontamination using liquid sporicides affects a reduction in the number of aerosolized spores in an enclosed volume is a research gap.

Hypochlorous Acid.

In aqueous solution, hypochlorous acid (HOCl) exists in equilibrium with its conjugate base, hypochlorite. Together these two species are referred to as free available chlorine (FAC). Chlorine bleach, an aqueous solution of sodium hypochlorite, is the most common source of HOCl in studies of *B. anthracis* inactivation on building and environmental surfaces. The protonated HOCl is the more effective sporicide; for surfaces treated with a constant FAC, acidifying to pH≤ 6 increases sporicidal effcacy.[62] As with other oxidizing compounds (e.g., ClO_2, peroxides, ozone; discussed below) the spore killing mechanism of hypochlorite is thought to involve spore inner membrane damage, with spore coat protein offering resistance.[58,59]

pH-Adjusted Bleach.—As summarized in Table 1, spray-applied solutions of acidified bleach (pH-adjusted bleach or pAB) with FAC levels ranging from 5000 to 7000 ppm (ppm) were effective (>6 LR) against *B. anthracis* on nonporous building surfaces, although effcacy on porous surfaces varied by material.[34,51,52,63] Effcacy on porous surfaces may be limited by consumption of FAC through oxidation of organic materials (which often comprise porous surfaces) or by low surface wettability.

A large field study effectively used spray-applied pAB against B. atrophaeus on a variety of nonporous building surfaces,[64] although the porous materials including ceiling tiles, furniture, and carpet were bagged and removed from the building and treated as waste. Some

of these porous items are good candidates for immersion in pAB. Low effcacy against *B. anthracis* on carpet, for example, can be overcome through immersion of the carpet in pAB for at least 30 min.[65]

Although chlorine bleach is corrosive, no damage was observed to building material coupons assessed in a laboratory study,[34] but during a field-scale application, wood-laminate floor was damaged.[64] While the aforementioned studies typically used acetic acid (such as vinegar) to lower the pH of the bleach solutions to a range of 6−7, Frazer et al.[66] demonstrated that acidified bleach was equally effective when using HCl for acidification.

Hypochlorite Solutions Diluted with Water, no pH Adjustment.—These solutions were effective against *B. anthracis* (Ames and other strains such as Vollum and Albia) on nonporous surfaces immersed in the liquid (3000−5000 ppm FAC),[31,67] but effcacy was reduced or required substantially higher concentrations in the presence of organic burden.[68–70] Several spray-applied commercial off-the-shelf cleaning products containing bleach (≥20 000 ppm FAC or 2% hypochlorite) and surfactants, with pH of approximately 12.5, were effective against spores of B. atrophaeus on porous and nonporous building surfaces[71] The use of carbon nanotubes improved effcacy of hypochlorite solutions as well.[72] Diluted bleach and consumer products may be less hazardous to work with (compared to pAB) because less chlorine gas is released from the liquid at higher pH, potentially reducing the level of respiratory protection needed. Chlorine gas workplace exposure limits range from 0.1 to 1.0 ppm by volume (ppmv).[73]

Sodium Dichloroisocyanurate.—An alternative method of producing HOCl in solution is the dissolution of commercially available tablets or powders of sodium dichloroisocyanurate (NaDCC; e.g., for swimming pool disinfection). NaDCC hydrolyzes to form FAC, monochloroisocyanurate, and isocyanurate in equilibrium. At near neutral pH, approximately half the available chlorine exists as mono- or dichloroisocyanurate.[74] This equilibrium between chloroisocyanurates and FAC is the suggested cause of the enhanced microbiocidal effcacy of NaDCC under organic burden compared to the effcacy of bleach.[75] As FAC is consumed by reaction with organic material, it is slowly replenished by hydrolysis of the chloroisocyanurates. The continued release of FAC provides a low concentration of FAC over an extended period. In the case of bleach, all the available chlorine present in solution is FAC that may be consumed immediately by reaction with organic material. Examples of where NaDCC offers increased effcacy on organic materials compared to pAB or dilute bleach are found here.[34,51,76] Despite these few studies, the FAC levels and CTs needed for effective use of spray-applied NaDCC against *B. anthracis* on porous and nonporous surfaces is a research gap.

Application Methods.—In addition to application as a spray, many of the above-mentioned HOCl-based decontaminants are also effective as fogs, wetted wipes, or gels. Fogging with pAB, diluted bleach, or NaDCC was effective (>6 LR) against spores of *B. atrophaeus* on nonporous surfaces but only moderately effective or ineffective on most porous materials (Table 1).[77] Several commercial off the shelf bleach-wetted wipes were effective (>6 LR) against *B. atrophaeus* on nonporous surfaces. Acidified bleach mixed into a polymeric gel was effective against *B. anthracis* Vollum on painted steel.[78] This gel was

able to adhere to vertical surfaces, potentially providing greater wetted CT per application than a spray-applied liquid.

Electrochemical Generation.—Hypochlorous acid can also be generated electrochemically from an aqueous solution of NaCl.[79–81] In an electrolytic cell, chloride ions are oxidized at the anode to form Cl_2 gas, which reacts to form hydrochloric acid and HOCl (a solution referred to as anolyte water).[82] The primary advantage of this system is the generation of HOCl on site without the need to transport or store large volumes of bleach. The effcacy of electrochemically generated HOCl is similar to that of pAB: effective against *B. anthracis* on tested nonporous surfaces (>6 LR) but only moderately effective or ineffective on tested porous surfaces.[83]

Calcium Hypochlorite.—Refer to the SI for further information and data on this source of HOCl sporicidal chemistry.

Peroxide Compounds.

Although there are several commercial decontaminants containing hydrogen peroxide (HP) combined with additional ingredients (e.g., surfactants, chelators, activators, and catalysts), aqueous HP alone may be effective on materials only when used at relatively high concentrations. From the United States Environmental Protection Agency's (USEPA) list of registered antimicrobial products used as sterilizers, the minimum concentration of aqueous HP when used without additional ingredients is 35%.[84] Other studies also confirm the need for relatively high concentrations when using HP by itself.[70,85,86]

H_2O_2 with Activators or Catalysts.—The effectiveness of aqueous HP can be improved by adding bleaching activators such as glycerol diacetate (diacetin) or catalysts such as bicarbonate and molybdate ions. Bleaching activators are a class of compounds with O- or N-bound acetyl groups that react with HP to form PAA.[87] For example, tetraacetylethylenediamine combined with sodium perborate (both compounds may be found in laundry detergents; the latter dissociates to HP in aqueous solutions[87]) was shown to have antimicrobial activity.[88] Further, diacetin is cited as the activator in a patent[89] for a two-component product currently sold as Easy Decon 200. Triacetin and potassium carbonate were added to HP solution in a decontamination study using B. atrophaeus spores, to produce an activated HP solution that was shown to be effective on a number of mostly nonporous materials when applied as a spray, but was ineffective on wood and concrete.[90] Bicarbonate and molybdate ions catalyze the oxidative reactions involving HP by forming peroxymonocarbonate (HCO^-_4) and peroxomolybdate intermediates, respectively, which are attributed to increased oxidation rates of organic compounds.[91–94] Decon Green, a formulation developed by the military, contains both bicarbonate and molybdate catalysts.[95] These amended or activated formulations of HP can be effective at lower concentrations than the concentrations required in unamended solutions. One study found that spray-applied EasyDecon 200 (<4 wt % HP) and Decon Green (<35 wt % HP) were effective against *B. anthracis* (>7 LR) on a variety of outdoor building materials (Table 1), but not on the organic materials treated wood or asphalt.[34]

Peracetic Acid.—Products utilizing PAA chemistry are generally some of the most effective liquid sporicides that are commercially available (hospital/medical instrument applications), and are produced in equilibrium with acetic acid and HP. Against a suspension of B. atrophaeus spores, PAA was substantially more effective (several orders of magnitude) than HP.[96] Leggett et al.[97] confirmed the synergistic activity of the combination of PAA and HP via suspension tests using *B. subtilis* spores, and hypothesized that aqueous HP weakens the spore coat, but that inactivation is primarily due to PAA. As noted in Table 1, spray-applied formulations of PAA (0.08−0.5 wt %) were effective against *B. anthrac* is on several indoor and outdoor building materials, but notably ineffective on unpainted concrete.[34,51,98,99] PAA has also been evaluated via application with wipes[100] and fog.[101] In a comprehensive study, fogging of PAA (and also aqueous HP) was found to be effective on most of the subway railcar materials that were tested, in at least one of the conditions evaluated.[102] Like the spray-applied tests, however, fogging of PAA was ineffective on unpainted concrete. The reduced effcacy of HP-based sporicides on unpainted concrete is thought to be related to the decomposition of HP caused by chemical interactions with this material (e.g., material demand).

Solid powders of peracetyl borate that dissolve in water to form PAA have been suggested as less hazardous and more economical to transport than PAA. A commercial formulation of peracetyl borate was effective (>6 LR) against *B. anthracis* spores on a variety of naval equipment surfaces, using an immersion test approach.[54] Further evaluation of peracetyl borate and development of other solid precursor materials for PAA is recommended.

Other Noteworthy Peroxides.—Sodium persulfate, also referred to as sodium peroxodisulfate, when activated produces highly reactive but persistent sulfate radicals, and is used com-mercially as an oxidant to treat organic contaminants in soil[103] and groundwater[104] and has been tested for inactivation of microbes.[105] In a systematic study involving several test materials and other parameters, sodium persulfate activated with 8% aqueous HP was effective in inactivating spores of *B. anthracis* on variety of difficult-to-treat porous and organic outdoor sur-faces including asphalt, brick, soil, and concrete (Table 1).[63,106] There are several activators other than HP that can be used with sodium persulfate[105] to produce sulfate radicals, and this area is suggested for further R&D.

A few studies evaluated aqueous solutions or gels of potassium peroxymonosulfate ($KHSO_5$) by dissolving the commercially available salt $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$ (Oxone, DuPont, Wilmington, DE).[107] Addition of sodium chloride to buffered solutions of peroxymonosulfate results in the formation of HOCl, which greatly increased the effcacy of 100-g/L Oxone against a suspension of *B. atrophaeus*.[108]

Aqueous Chlorine Dioxide.

Due to its volatility and potential to degrade during storage,[109] most aqueous chlorine dioxide (ClO_2) solutions tested for effcacy against *B. anthracis* spores on building or environmental surfaces are prepared at the point of use. Methods of generating ClO_2 include mixing solutions of sodium chlorite and bleach under acidic conditions, the use of

commercial products containing sodium chlorite and activating compounds that react when mixed in solution, and electrochemical generation from sodium chlorite.

Spray-applied solutions of ClO_2 at measured concentrations of 3000−4000 ppm were effective against *B. anthracis* on several nonporous building surfaces but ineffective or not consistently effective on porous surfaces and soils (Table 1).[110] Spray-applied commercial products with lower ClO_2 concentrations ranging from 200 to 1500 ppm (as reported by their vendors) have been mostly ineffective (<2 LR) on porous and nonporous surfaces.[51,52]

A separate study found that if porous materials (carpet and particle board) were immersed in a 1000 ppm solution of ClO_2 at >6 LR of *B. anthracis* was achieved.[65] Improved effcacy through immersion at a lower concentration suggests that the limited effcacy of spray-applied liquids could be due to spray-application parameters (e.g., droplet size or insuffcient number of applications). Although not yet substantiated, authors have also suggested that ClO_2 in the aqueous phase could be lost through volatilization from spray droplets or from the wetted surface during the application.[52,111]

Several aqueous solutions of ClO_2 in the range of 5000− 6000 mg/L were produced via an easy to use commercially available product that utilizes sodium chlorite and sodium bisulfate. When applied as a fog, the ClO_2 solutions were effective on a number of materials.[77] Further investigation of this simple ClO_2 generation technology and application approach is warranted. In addition to chemical methods, aqueous ClO_2 can be generated electrochemically using solutions of sodium chlorite and sodium bromide.[112]

Aldehydes.

The mechanism of spore killing by this chemistry is thought to be DNA and germination apparatus damage.[59] While aqueous solutions of formaldehyde (e.g., formalin) are sporicidal, Spotts Whitney et al.[2] suggest that the classification of formaldehyde as a possible carcinogen has limited its use. Further, the European Union classifies formaldehyde as a Category 1B carcinogen, and has approved its use as an antimicrobial only under certain conditions.[113] Liberal applications of 5−38 wt % solutions were used to decontaminate Gruinard Island, a former site of *B. anthracis* (unspecified strain) weapons testing in Scotland, resulting in no detectable spores.[114] No controlled laboratory studies reporting the effectiveness of aqueous formaldehyde against *B. anthracis* on surfaces or soil could be located.

Table 1, below, is a synthesis of the more pertinent test conditions and results for liquid-based decontaminants.

Other liquid sporicides of note, including glutaraldehyde and aqueous solutions of ozone, may be found in the SI.

GASEOUS DECONTAMINANTS

Gaseous decontaminants are generally used for enclosed volumes such as buildings, although tarpaulins may be used to contain the gas during decontamination of soil or other complex surfaces. With adequate air mixing, sporicidal chemicals in the gas-phase have the

advantages (over liquid decontaminants) of inactivating aerosolized spores, can be widely dispersed, and can penetrate through cracks and crevices to decontaminate hard to reach surfaces. As with liquid decontaminants, gaseous decontaminants are generally more effective at higher concentrations, temperatures, and CTs. In addition to these parameters, relative humidity (RH) is also an important environmental factor in gas-based decontamination, with effcacy generally improving with increasing RH. Generating, achieving, and maintaining suffciently high concentrations of the sporicidal gas needed for effective decontamination inside a building or volume to be decontaminated tends to be a technical challenge. R&D is therefore moving toward finding effcacious conditions at relatively low concentrations coupled with longer CTs. The use of lower concentrations may also have the added benefit of improved compatibility of materials (research is needed to confirm this benefit), less hazardous operating conditions, and allowing more vendors to provide decontamination services in the event of wide area *B. anthracis* spore release. Lastly, all sporicidal gases are typically hazardous themselves, so there may be a need to minimize the release of the gas during or after decontamination via containment or capture techniques.[115] Table 2, below, provides a synopsis of conditions and results for gaseous decontaminants. As with Table 1 for liquids, this table is not intended to be inclusive of all data, but rather is intended to provide a synopsis of some of the more relevant effcacy data.

ClO_2 Gas.

Following the Amerithrax incident, four buildings were decontaminated using gaseous ClO_2.[116] Chlorine dioxide gas has also been used in several other *B. anthrac* is contamination incidents, such as the decontamination of a village hall in Scotland.[10] Since these incidents, a substantial body of R&D in the use of this gas as a sterilant has been produced, and most of these studies show the gas to be a highly effective decontaminant. However, because of stability issues (i.e., unable to be compressed and stored), ClO_2 gas must be generated at the point of use.[117] Other issues with ClO_2 gas, related to its detrimental interactions with materials (e.g., material demand, corrosivity, and formation of unwanted byproducts) have also been raised.[118]

ClO_2 gas can be generated via a wet or dry chemical process, although no difference in decontamination effcacy was observed as a function of the generation technique.[6] Over the past 15 years, decontamination effcacy testing with ClO_2 gas has been conducted with numerous types of materials under a variety of operational and environmental conditions. Earlier tests with ClO_2 gas were conducted mostly at relatively higher levels of the gas (i.e., >1000 ppm), and have demonstrated its generally high effcacy on numerous materials,[6,119,47] including more difficult materials such as soils[120,121] and grimyconcrete material obtained from a subway tunnel.[122]

In transitioning the research to tests with relatively lower levels of ClO_2, associated with longer CTs, a 200 ppm level (75% RH) for 4 h was shown to be effective in inactivating spore populations of *B. subtilis* in galvanized metal ductwork from a mock heating, ventilation, and air conditioning system.[123] Other studies have demonstrated the conditions required for effective decontamination using ClO_2 gas levels ranging from 350 to 750 ppm.[124–126] In a series of six small-chamber experiments conducted at either 100 or 200 ppm of

ClO_2 (75% RH, 24 °C; CTs ranging from 2 to 12 h), several building materials were effectively decontaminated in every test except for moderate decontamination effcacy of the wood coupons at 200 ppm.[127]

With respect to the effect of temperature and RH, in a study simulating the relatively cooler temperature that may be encountered in a subway system (11 °C), the lower temperature greatly diminished the decontamination effcacy[128] In this same study, lowering RH from 75 to 50% (at 24 °C) also greatly reduced effcacy. Wang et al.[129] confirmed that increasing concentration and RH significantly improved inactivation of *B. atrophaeus* spores on paper, with rapid improvement occurring when RH > 70%.

Hydrogen Peroxide Vapor.

Hydrogen peroxide vapor (HPV) is a well-established antimicrobial technology that has been described in patents since 1934[130] and has been extensively investigated for its ability to inactivate all classes of microorganisms,[131] Because of its benign decomposition products (O_2 and H_2O), HPV is increasingly being used in place of other gas-phase sterilants such as ethylene oxide and formal-dehyde.[132] HPV has also been shown to be more compatible with electronics and other materials compared to sporicidal gases such as ClO_2.

Since 2002, the use of HPV as a sporicide, and for B anthracis inactivation in particular, has evolved from use as a small chamber sterilant for medical devices to its application as a volumetric or building decontaminant. HPV was used to decontaminate two large buildings contaminated with *B. anthracis* spores following Amerithrax.[116] In the fumigation of one of the contaminated buildings (Department of State SA-32), special efforts were required and difficulties were encountered to reach and maintain the target HPV concentration.[14] In the full-scale field evaluation of HPV as part of the Bio-Response Operational Testing and Evaluation study,[64] roughly one-third of the postdecontamination samples were positive for spores of the target organism Bacillus globigii. The poor results were similarly attributed to not being able to meet the target concentration of 250 ppm throughout the building.

Recent research on HPV has sought to elicit the effect of several variables on the effcacy of HPV, including material. Such studies[48,65] have demonstrated that building materials such as wood, unpainted concrete, and carpet are more difficult to decontaminate with HPV compared to nonporous materials such as glass, laminate, and galvanized metal. Wood et al. [131] confirmed that unpainted concrete was decontaminated ineffectively but that decontamination was effective for the majority of indoor materials and conditions that were tested. As with HP-based liquid sporicides, HPV's poor decontamination performance on unpainted concrete is likely due to this material's high demand for the HPV.[133]

The use of a proper surrogate for *B. anthracis* in HPV decontamination studies has also been investigated.[48,134] For example, while G. stearothermophilus may be the internationally recognized biological indicator organism for HPV,[134] Rogers et al.[48] have shown that B. subtilis may be a more representative organism to model *B. anthracis* resistance to inactivation by HPV. Other studies have investigated the effect of spore loading[36] and the inactivation of different spore producing species such as *C. difficile*[57] and *C. botulinum*.[135]

As summarized by Unger-Bimczok et al.,[132] disagreement remains in the literature as to the effect of RH on decontamination effcacy and whether condensation on surfaces should occur or be avoided with the use of HPV. They report that it is common procedure to dehumidify the volume to be decontaminated to avoid condensation of HPV, while others claim that condensation is critical to effective decontamination with HPV. For example, Pruss et al.[136] claim that a lower surface temperature enhances condensation, and that this improves effcacy, although only for certain species of bacteria. UngerBimczok et al.[132] showed that increasing RH improves decontamination effcacy for *G. stearothermophilus* spores (due to increased condensation), but this effect is more pronounced when decontaminating with relatively lower concentrations. In addition, they conclude that the molecular deposition of water and HP on surfaces is more important than the HPV concentration for effcacious microbial inactivation.

Because of the large volume of literature on the use of HPV as a sterilant, along with a myriad of experimental conditions, there is considerable variability in the concentrations and CTs (and thus dose, i.e., concentration × CT) that have been studied and recommended for effective inactivation of bacterial spores. Most of the studies have demonstrated the use of HPV at concentrations over 200 ppm, with associated CT on the order of minutes to a few hours. One vendor requires their HPV technology to achieve concentrations between 250 ppm to 930 ppm.[137] More recently, however, R&D is moving toward simplifying the fumigation process via using lower HPV concentrations, coupled with longer CTs, than are typically used or suggested by vendors of HPV generating technology.[138–140] Wood et al.[131] showed that relatively low HPV concentrations (average of 5−10 ppm), produced simply via the use of inexpensive, commercially available humidifiers and 3−8% aqueous HP solutions, create sporicidal conditions on many different materials after a few days' CT. Concrete was the exception, that is, it has been shown in other studies to be poorly decontaminated using HPV.[36,131]

Methyl Bromide, Methyl Iodide, and Metam Sodium.

Methyl bromide (MeBr), methyl iodide (MeI), and metam sodium are all fumigants that are currently used or have been used as pesticides for soils (agriculture), food commodities, and/or structures. MeBr and MeI are alkylating agents (like EtO), which kill spores via DNA damage.[59]

Methyl Bromide.—MeBr was recognized in 1950 as sporicidal for *B. anthracis* spores.[141] In the event of a wide-area release of *B. anthracis* spores, MeBr has several advantages as a decontaminant including an existing industry with personnel experienced in its use; MeBr easily penetrates materials and is relatively compatible with materials. While it is being phased out under the *Montreal Protocol on Substances That Deplete the Ozone Layer*, several million kg of MeBr are still being used in the U.S. annually under quarantine, preshipment, and critical use exemptions.[142] Global quarantine and preshipment use of MeBr in 2013 is estimated to be 10,000 t.[143] To mitigate impacts to the stratospheric ozone layer, MeBr can be captured with activated carbon following its use as a decontaminant.[144]

Since Amerithrax, several laboratory and field studies have been undertaken to further understand the sterilant properties of MeBr. Juergensmeyer et al.[145] conducted a study using spores of several microorganisms (*B. anthracis* ANR-I, *G. stearothermophilus, B. atrophaeus, and B. thuringiensis*) inoculated onto glass slides, with experiments conducted at 37 °C and varying MeBr concentrations. No *B. anthracis* spores were recovered (>7 LR) after a 48 h exposure to 80 mg/L. In another laboratory study with MeBr tests at 37 °C and 75% RH, greater than 6 LR was observed for *B. anthracis* spores on all materials tested except cellulose.[65] In these tests, B. subtilis was much more resistant to MeBr than *B. anthracis*, a surprising finding.

From a comprehensive parametric study, Wood et al.[142] reported on the required CT needed to achieve >6 LR of *B. anthracis* spores on the six building materials tested in their study, as a function of multiple fumigation conditions (varying RH, temperature, and concentration). As an example, 18 h CT was required with a MeBr concentration at 300 mg/L, 27 °C, 75% RH. MeBr was also found to be effective against *B. anthracis* spores in 1 cm depth topsoil at 25 °C (180 mg/L; CT of 36 h; RH > 75%).[63]

MeBr has been demonstrated in field-scale tests, such as with a 1,444 m^3 building in which no damage to the building or its contents was observed.[146] A full-scale field demonstration for decontaminating a subway railcar using MeBr was also successfully conducted.[147] With the thought that MeBr could be used for decontamination of underground transportation systems (e.g., subway tunnels), tests were conducted at lower temperatures to assess the potential.[148] MeBr was found to be effective in inactivating spores of *B. anthracis* Sterne on several tunnel materials at 10 and 4.5 °C, with extended CTs of 4 and 7 days, respectively.

Methyl Iodide.—Although no longer used or manufactured in the U.S., the pesticide MeI is used in several other countries as an alternative to MeBr.[149] In one laboratory study with MeI, several conditions (100−400 mg/L) were found to be effective in inactivating *B. anthracis* Ames spores on all of the materials tested.[150]

Metam Sodium.—Lastly, metam sodium is the most widely used soil fumigant in the US and was effective in decontaminating topsoil (*B. anthracis*) and a test dust under a number of test conditions.[63] Metam sodium reacts with moisture in the soil to produce methyl isothiocyanate gas, the chemical responsible for biocidal activity. Indeed, adding moisture to the soil was found to improve sporicidal activity against *B. anthracis* spores.

Formaldehyde Gas.

Formaldehyde gas has been used as a decontaminant and sterilant for over 100 years.[151] It has been commonly used for decontamination of high efficiency particulate air filters, biological containment laboratories and safety cabinets, and animal housings, due to its effcacy, low cost, and material compatibility.[152] Formaldehyde gas was used to decontaminate mail sorting equipment from a postal facility in Landover, Maryland.[153] However, due to concerns over its toxicity, alternatives for the above-listed applications are being explored.[154]

Gaseous formaldehyde can be generated via the heating of paraformaldehyde (PF), a mixture of formaldehyde-based polymers that is solid at room temperature.[155] The heating of PF can occur simply through the use of a hot plate[156] or through a controlled process using commercially available equipment.[49,152] Gaseous formaldehyde may also be generated via the heating of formalin, an aqueous mixture of formaldehyde (typically 37% by weight) and methanol. As with PF, formalin can be heated by simple means such as a wok[154] or via more sophisticated and controllable equipment.[157] Ngabo et al.[158] recently investigated the effcacy of formaldehyde vapor generated using formalin, at several concentrations and exposure times, and reported that 4.2 mL formalin/m^3 with a six h CT, was effective in inactivating spores of *B. atrophaeus* on stainless steel.

With respect to an effective or optimal formaldehyde gas concentration, 10.5 g solid PF/m^3 of volume to be decontaminated has been recommended by various organizations[159,160] and has been used in various studies.[49,152,153] This level was originally recommended by Taylor et al.[161] as a result of their investigations. Note that this concentration is the original amount of solid PF to begin with prior to sublimation and is not the actual concentration of formaldehyde in air. In fact, very few decontamination studies have actually measured and reported levels of formaldehyde gas in the air, with the exception of Rogers et al.[49] and Ackland et al.[162] The latter reported that the vapor phase equilibrium concentration of gaseous formaldehyde at 20−21 °C is 2 g/m^3, and when exceeding this concentration, condensation of formaldehyde occurs. Indeed, in the study by Rogers et al.,[49] the original amount of solid PF was equivalent to 10.5 g/m^3, but actually measured only 1100 ppm formaldehyde (= 1.36 mg/L at 23 °C) in the air.

With formaldehyde condensation, it is common practice to employ ammonia gas to react with the formaldehyde to produce the relatively benign byproduct methenamine, a solid residue that forms on surfaces and is typically removed for practical reasons. Ammonia gas can be generated by heating ammonium carbonate or ammonium bicarbonate.[156] Further research is needed to determine effective gas-phase formaldehyde levels that minimize methenamine production.

Spiner[163] originally demonstrated that decontamination effcacy with formaldehyde gas is improved at elevated RH levels, and consistent with this finding, more recent studies have employed RH levels in the range of 70−90%.[49,152] Rogers et al.[49] demonstrated effective decontamination against *B. anthracis* on numerous building materials. We were unable to locate other formaldehyde gas decontamination data for *B. anthracis* nor with its use on realistic building materials.

Ozone.

The use of ozone gas as a biodecontaminant has been explored since 1982.[164] While the use of ozone gas holds promise, it has not been demonstrated full-scale.[14] Nevertheless, gaseous ozone can be generated in large quantities (up to 300 kg/h by one vendor) for use in water utilities and other industrial water and wastewater applications,[165] and approximately 10% of U.S. water treatment plants use gaseous ozone.[166] is gaining traction for use in healthcare environments for disinfection and sterilization,[167–170] and has been approved for reprocessing (sterilization) of medical equipment that cannot be heat-treated.[171]

Mahfoudh et al.[172] discuss the role that elevated RH plays in fumigation with ozone and other gaseous decontaminants, via the swelling of spores and creating “channels” for gas to diffuse into the spore. Other research groups[50,173–175] have corroborated the finding that increasing RH levels (>80%) improves effcacy with ozone gas.

Using ozone gas at relatively low levels (1−25 ppm), Akbas et al.[176] and Sharma et al.[169] demonstrated moderate effcacy (2−4 LR) against spores of B. cereus and C. difficile on a number of different materials. With higher ozone concentrations, Aydogan et al.[175] reported 2−4 LR (depending on material) in B. subtilis spore populations when decontaminating with ozone at approximately 5000 ppm, 90% RH for 4 h. In a large comprehensive study with numerous experiments utilizing spores of both *B. anthracis* and *B. subtilis*, effective decontamination with ozone gas was achieved at 85% RH, with required ozone levels ranging from 9800−12 000 ppm, depending on the material.[50]

PHYSICAL-BASED DECONTAMINANTS

Thermal Treatment.

Thermal treatment techniques for the inactivation of microorganisms have been used for millennia,[55] and may be categorized as either wet or dry heat. Wet heat includes environments with air at elevated temperatures and saturated with moisture (100% RH) or boiling water.[177] Autoclaves are an example of “wet heat”, where steam is used at elevated pressures and temperatures.[178] While sterilization via autoclave is a ubiquitous technique except where there are material compatibility issues,[177] studies have shown that under certain circumstances, typical autoclave operation for waste treatment may not completely inactivate spore populations.[178,179] Protein damage is the likely spore inactivation mechanism for wet heat, while DNA damage is the major mechanism with dry heat.[8,59]

A dry heat environment is characterized with an RH level <100%, such as the environment found in an incinerator,[180] an ampule in an oil bath, or infrared heating.[181] Spores are generally more resistant to dry heat than wet heat.[177] Peeler et al.[182] showed that with dry heat (113 and 125 °C), inactivation of *B. atrophaeus* spores improved with increasing RH levels, except at RH levels less than 10%. More recently, Buhr et al.[55,183] showed that in dry heat environments, increasing RH may be accompanied by an increase in effcacy, but their results were somewhat confounded by other test variables.

While research continues to further elucidate the mechanisms associated with wet[184–186] and dry heat[8,181,187] spore inactivation, thermal treatment applied on a large scale may be impractical.[14] Nonetheless, Buhr et al.[188] demonstrated decontamination of a C-130 aircraft using dry heat (75−80 °C, 70−90% RH, 7-day CT). While 89% of surface samples were negative for the test organism *B. thuringiensis*, overall effcacy was estimated to be >7 LR; all BIs and inoculated coupons were completely inactivated. No information was provided relative to impact of the dry heat treatment on aircraft materials.

UVC-254.

The germicidal effects of ultraviolet radiation (UV) have been known for 150 years, and the majority of papers published on the effects of UV on bacteria have focused on UV with a

wavelength of 254 nm, which is produced via low pressure mercury vapor lamps.[189] We refer to this wellestablished sterlization technique as UVC-254. The wavelength range for UVC is 190−290 nm; germicidal UVC produced from sources other than mercury vapor bulbs (e.g., light emitting diodes) is discussed in the SI section on emerging decontamination techniques. Medium pressure mercury lamps produce UV light over a broader range of wavelengths (200− 400 nm).[190] UVC inactivates bacterial spores via damage to DNA.[8,59,189]

In a review of UVC-254,[189] the authors conclude that "the literature revealed that many studies lack information on dosimetry, microbial quality, or experimental details that would allow comparative analysis of UVC data", and we would agree with this assessment. In the cases where dosimetry data are reported, experimental procedures range widely in terms of the fluence rate, dosage, exposure time, the distance between UVC source and microbial population, and techniques for measuring UVC. For example, at the low end of the dosage range, Menetrez et al.[191] report that 17.5 mJ/cm^2 achieved a 59% reduction of *Bacillus anthracis* Sterne spores on Agar plates. At the upper end of the range, Kesavan et al.[192] reported that a dose of 2300 mJ/cm^2 provided a 2−3 LR.

While several studies report bacterial spore population inactivation kinetics, none of the literature reports UVC-254 dosages required to achieve effcacious decontamination, that is, ≥ 6 LR. That UVC-254 may be unable to achieve greater than 4 LR on materials could be due to a shielding or shading effect at the microscopic level.[189] This shielding or shading may be due to effects of materials[189] or from agglomeration of spores.[192] Further, several studies have reported a tailing effect,[189,192–194] whereby an initial 1−2 LR of spores occurs rapidly at low UVC-254 dosages, but with minimal additional decay after prolonged exposure. For example, Owens et al.[193] showed that ~4 LR of *B. anthracis* Sterne was achieved with 1000 mJ/cm^2 using a medium pressure Hg lamp, but that no further reduction occurred thereafter, up to a dose of 4000 mJ/cm^2.

Most of the studies on UVC-254 for bacterial spore inactivation conducted over the past decade focused on development of additional dosimetry data for different materials. As with many other inactivation studies, the scientific literature for UVC-254 is typically lacking in decontamination effcacy data for materials other than rudimentary laboratory substrates or environmental matrices such as glass slides,[195] filter paper,[196] water or liquid suspension,[194,197] air,[198,199] or agar plates.[200] Further, many of the UVC-254 data in the literature are for vegetative bacteria or spores of *B. subtilis*,[201,202] *B. atrophaeus*,[199] *B. cereus*,[203] or *B. anthracis* Sterne;[191,193,203] we were unable to find any UVC-254 dosage/effcacy data for virulent forms of *B. anthracis* spores. Other gaps in the literature include the effect of RH and fluence rate on effcacy with UVC-254, as well as effcacy for other UVC sources.

CONCLUSIONS AND RESEARCH RECOMMENDATIONS

Substantial progress has been made in the past 15 years in the science and technology of inactivating spores of *B. anthracis* and other bacteria. One of the more significant advancements has been the development of a large body of data which demonstrates decontaminant operational (e.g., chemical concentration, CT, dosage requirements) and

environmental conditions (temperature, RH) required for effective killing of spores. Further, we have elucidated the chemical mechanisms of decontaminants and (related to this) learned that the building material or environmental media that the spore population is associated with has a significant effect on decontamination effcacy. While most of this research was conducted at bench scale, transitioning decontamination technology to increasingly larger scale is a current trend that will need to continue, to uncover issues of a more operational nature. For example, several technologies that were suggested years ago as having potential[2,14] have since been demonstrated to be effective at larger scales (one case in point being MeBr).

For *B. anthracis* in particular, research has been focused on developing the capacity to respond to an intentional, wide area release of such spores. Since the availability of chemicals, personnel, and expertise will likely impact the decontamination effort after a wide-area incident, having numerous techniques available to decontaminate buildings and other environments would be advantageous. This implies developing more simple, readily available but effective methods, such as disseminating HPV through a humidifier,[131] using off-the-shelf cleaning products found to be sporicidal;[71] or using agricultural pesticides found to be sporicidal (such as MeBr), in which there is an existing industry. Research and development is expected to continue in this vein. Similarly, transfer of sterilization techniques used in the medical and food industries to biodefense applications is a current trend that will continue. Many of the techniques discussed in this review for killing *B. anthracis* can also be effectively used for *C. difficile*, and vice versa.

Additional research is needed to investigate technologies that are effective against spores of *B. anthracis* or other *Bacilli* in suspension or as an aerosol but have not been fully evaluated on relevant building or environmental surfaces. Emerging decontamination techniques (e.g., atmospheric cold plasma) need to be further developed to make them commercially viable and feasible for use at a larger scale. More research is also needed to identify effective techniques for challenging conditions such as low temperatures, and complex, outdoor materials such as soil and vegetation. Most laboratory studies are conducted at relatively high spore loadings to demonstrate a >6-LR; identification of less aggressive methods that achieve complete inactivation but at lower spore loadings will be useful. Lastly, additional efficacy data are needed for strains of *B. anthracis* that have been often overlooked in research.

In closing, please refer to Table 3 which provides a qualitative summary of the techniques discussed in this article, in terms of their advantages, disadvantages (e.g., material compatibility issues), and other noteworthy items (e.g., full-scale usage or demonstration).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Decontamination Effcacy[a] Synopsis for Liquid Sporicides[b] under Selected Conditions and Materials[c]

Liquid	Approx. concn.[d]	Apps.[e]	Contact time[f]	Strain[g]	Glass	Metal[h]	Hard tile[i]	Lam	PWP	Concr	Brick	Asph	Carp	Ceil tile	Bare wood	Soil	Reference
pAB	6500	1-2	10-60	Baa													51, 52, 204
pAB[j]	6500	1-2	Dried	Bg[k]													205-207
pAB	6500	4	30-60	Baa													34, 98
pAB	6500	Imm.	60	Bg													208
pAB/DB	65-20 x10^3	Fog	0-20[L]	Bg[k]													77
NaDCC	48-85[m]	1-2	30-60	Baa													34, 51, 98
NaDCC	25000	Fog	16-21[L]	Bg[k]													77
ClO_2	1500	5	70	Baa													51
ClO_2	2500	2-4	60-120	Baa													110
ClO_2	1000	Imm.	120	Baa													65
ClO_2	6000	Fog	19[L]	Bg[k]													77
HP	25000	4	5	Bs													86
EasyDec	< 4000[n]	3-6	30-60	Baa													34
DecGrn	< 35000[n]	2	60	Baa													34
PAA	5000	1,12[o]	10-60	Baa													51, 98
PAA	800-2200	2-6	30-60	Baa													99
PAA	45000	Fog	2-19[L]	Bg[k]													101
Activated persulfate	120 x10^3	3-6	67-167[L]	Baa													63, 106
L-Gel	300[p]	1	Dried	Bg[k]													107

[a] Effcacy: Horizontal green lines LR ≥ 6 or complete inactivation; Orange trellis, 6 > LR ≥ 3; complete red fill, LR < 3; no fill, not tested; vertical, mixed results.

[b] DB, dilute bleach; EasyDec, EasyDecon; DecGrn, DeconGreen, other sporicide abbreviations defined in abbreviations section.

[c] Lam, decorative laminate; PWP, painted wallboard paper; Concr, concrete; Asph, asphalt; Carp, carpet; Ceil tile, ceiling tile.

[d] Concentration in mg/L unless otherwise noted; for pAB/DB and NaDCC, concentration is ppm FAC.

[e] Number of spray applications or Imm., surface was immersed in liquid; fog, surface was fogged with liquid.

[f] Contact time in minutes (unless otherwise noted) until surface was neutralized or sampled; dried, surface dried overnight before sampling;for fog applications, contact time is the time elapsed after fogging of liquid completed (dwell time).

[g] Baa, *B. anthracis* ames; Bg, *B. globigii/atrophaeus*; Bs, *B. subtilis*.

[h] Stainless steel, aluminum or galvanized metal.

[i] Granite, porcelain, or ceramic.

[j] Efficacy reported as surface log reduction (chemical inactivation and physical removal) with viable spores in neutralized rinsate/runoff and carpet vacuumed before treatment.

[k] Surface inoculated with aerosolized spores,

[L] hours,

[m] wt % concentration NaDCC in formulation.

[n] ppm of HP in formulation.

[o] Soil received 12 applications of PAA.

[p] g/L of Oxone in formulation.

Table 2.

Decontamination Effcacy[a] Synopsis for Sporicidal Gases[b] Tested under Select Conditions and Materials[c]

Gas	Concn[d]	RH[e]	CT[f]	Strain[g]	Glass	Galv metal	Steel	Al	Ceramic	Grnt	Lam	PWP	Concr	Carp	Ceil tile	Bare wood	Paper	Soil	Reference
ClO_2	3000	90	3	Baa															119
ClO_2	3000	75	5[h]	Ba[i]															6, 120
ClO_2	750	75	12	Bg															37
ClO_2	200	75	6	Bg															127
O_3	5000	90	6	Bs															175
O_3	9800	85	12	Baa															50
Form	1100	75	10	Baa															49
Form	4.2[e]	NR	6	Bg															158
HPV	1000	70	0.3[j]	Baa															48
HPV	500	NR	2	Baa															65
HPV	290	NR	3	Ba[i]															36
HPV	8.5	57	24	Baa															131
MeBr	212[k]	75	36	Baa															142
MeBr	110[k]	75[l]	18	Baa															65
MeBr	212[k]	75[m]	96	Baa															148
MS	160[n]	UC	336	Baa															63
Mel	105[k]	70	48	Baa															150

[a] Effcacy: Green horizontal lines, LR ≥ 6 or complete inactivation; Orange trellis, 6 > LR ≥ 3; Complete red fill, LR < 3; no fill, not tested; vertical, mixed results.

[b] Form, Formaldehyde; MS, metam sodium; other sporicide abbreviations defined in abbreviations section.

[c] Galv metal, galvanized metal; Grnt, granite; Al, aluminum; others as in Table 1.

[d] Concentration in ppmv unless otherwise noted.

[e] RH at ambient temperature (20−25 °C) unless otherwise noted; NR, RH and temp not reported but believed to be variable; UC, uncontrolled RH at ambient temp.

[f] Contact time in hours unless otherwise noted.

[g] Baa, *B. anthracis* ames; Bg, *B. globigii/atrophaeus*; Bs, *B. subtilis*.

[h] Topsoil tested at 2−4 h, 1 cm depth, using sterilized soil− see 2nd reference listed.

[i] Soil is Baa, others are Ba NNR1d1.

[j] Dwell time of 20 min with total cycle time (conditioning, gassing, dwell) of ~2 h.

[k] Concentration in mg/L air.

[l] Temp of 37 °C.

[m] Temp of 10 °C.

[n] 160 μL of 42.5 wt % metam sodium applied to small amount of soil in Petri dish.

Table 3.

Summary of Demonstrated Decontamination Techniques for the Inactivation of *B. anthracis* Spores[a]

technology	advantages	disadvantages	other notes
Liquid Sporicides			
acidified chlorine bleach (pAB)	readily available (chlorine bleach, vinegar), effective on many materials	less effective on organic materials, expected material compatibility issues, more prone to producing chlorine gas than diluted bleach without pH adjustment[77]	used in actual *B. anthracis* incidents[116] and in two field-scale demonstrations[64,209]
dilute chlorine bleach	readily available, COTS bleach cleaners with at least *2%* hypochlorite effective, effective on many materials	less effective on organic materials, expected material compatibility issues	used as a fog in field test[209]
NaDCC (dichlor)	readily available as swimming pool chemical; just add to	may leave residue,[77] gaps remain for determining efficacy on materials,	may be more tolerant of organic burden compared to other HOCl-based
	water, effective on many materials as CAS CAD	required concentrations—using swimming pool chemicals	decontaminants
electrolyzed water	generates HO Cl in situ, by passing electrical current through salt water	expect similar issues as PAB	reduces need to transport large volumes of bleach
aqueous hydrogen peroxide	benign decomposition products (H_20 and 0_2)	generally effective only at concentrations >35%, ineffective on unpainted concrete, less effective on wood	used in food industry
peracetic acid and related compounds	readily available, COTS, used in health-care and medical settings, effective on many materials	efficacy issues on unpainted concrete	most registered liquid sterilants use PAA or related chemistry as active ingredient,[84] can also be generated in situ with peracetyl borate and water
activated hydrogen peroxide	formulated on-site by mixing HP with activator; removes transport issues, would expect similar efficacy and issues as PAA, some COTS formulations available	efficacy issues on unpainted concrete	activators such as diacetin react with HP to produce PAA or related per oxygen compounds, military formulation uses bicarbonate molybdate catalysts
activated persulfate	effective on high organic materials, such as soil and asphalt, produces sulfate radicals which have capacity to overcome organic burden	strong oxidant, expected material compatibility issues; may be best suited for outdoor materials and soil	used full-scale for soil remediation with organic chemical contaminants, persulfate may be activated to produce sulfate radicals via HP, iron, and high temperatures
aqueous $C10_2$		mixed efficacy results; may require high concentration (>3000—4000 ppm) to achieve efficacy on some materials	typically generated at point of use, some COTS products available to ease generation of solution
Gases			
cio_2	generally effective on most materials	strong oxidant; material compatibility issues[45]	has been used full-scale in several actual *B. anthracis* incidents[10,116] and field tests,[64] fumigation at relatively higher concentrations requires higher level of expertise and generation technology, which are lacking
HPV	generally effective on many materials, compatible with most materials[45]	efficacy issues on unpainted concrete and possibly some organic materials such as carpet and wood	tests have shown that relatively lower concentrations coupled with longer contact times are effective, and may allow avoidance of expensive generation equipment,[131]

technology	advantages	disadvantages	other notes
			has been used full-scale in several actual *B. anthracis* incidents[116] and a field test[64]
MeBr	generally effective on most materials, highly penetrative of materials, compatible with most materials[45]	issues with supply of the gas, due to international treaty limiting production	demonstrated at several full-scale field tests[146,147]
Mel	effective on most materials tested	limited test data and information related to material compatibility	used as a structural fumigant in some countries, although not available in some countries
Metam sodium	useful/efficacious for soil decontamination	not tested against *B. anthracis* on materials other than soil	used widely full-scale as soil fumigant
formaldehyde	effective on most materials, compatible with most	typically neutralized with ammonia gas, which produces a residue that	has been widely used for BSL3 laboratory decontamination, but this is being phased out
	materials, inexpensive, easy to generate gas	must be removed	due to suspected carcinogenicity, used full-scale at postal facility[153]
ozone	efficacious on many materials at high concentrations and high RH	strong oxidant, expected material compatibility issues	used full-scale at many water treatment facilities, but not tested at full-scale as a high concentration fumigant
ethylene oxide	widely used in small-scale chambers for medical instrument and related sterilization	flammable, highly toxic, not available for use at large scale	
Physical			
wet heat	ubiquitous sterilization technique (e.g., autoclave), typically used for small items for medical/health fields, laboratories	some material compatibility issues, limited for use in relatively small chambers capable of withstanding pressure, although some commercial scale autoclaves available	
dry heat	may be more compatible to materials compared to wet heat	time and temperature requirements for efficacious conditions may be incompatible for several materials	tested full-scale on a military aircraft[188]
UVC-254	well-demonstrated technology, used commercially in several types of applications for room, surface, and air disinfection	no literature reports efficacy greater than 4 LR, which may be due to shielding/shading of materials and/or agglomeration of spores, may be better suited for nonporous materials	
Physical			
ionizing radiation	highly penetrative, efficacious on most materials, compatible with most materials, useful for decontamination of small, valuable personal items	like ethylene oxide and autoclaves, this technology confined to use in chambers, i.e., not available for large-scale, uses ionizing radiation source, which is hazardous and requires licensing requirements for use	fairly widely available technology, dosage requirements for effective decontamination vary by material; data gaps remain

[a]Decontaminants are considered effective for a material if demonstrate a LR ≥ 6, and/or complete inactivation of spore population. Further details on efficacy by material and other factors are found in Tables 1 and 2; COTS, commercial off-the-shelf.