

Toward a Consensus View on the Infectious Risks Associated with Land Application of Sewage Sludge

Emily Viau,[†] Kyle Bibby,[†] Tania Paez-Rubio,[‡] and Jordan Peccia^{*,†}

[†]Department of Chemical and Environmental Engineering Program, Yale University, Mason Laboratory, 9 Hillhouse Avenue, P.O. Box 208286, New Haven, Connecticut 06520, United States

[‡]Department of Civil, Environmental, and Sustainability Engineering, Arizona State University, Engineering Center G Wing, Room 252, Tempe, Arizona 85287, United States

S Supporting Information

ABSTRACT: The science linking processed sewage sludge (biosolids) land application with human health has improved in the last ten years. The goal of this review is to develop a consensus view on the human health impacts associated with land-applying biosolids. Pre-existing risk studies are integrated with recent advances in biosolids pathogen exposure science and risk analysis. Other than accidental direct ingestion, the highest public risks of infection from land application are associated with airborne exposure. Multiple, independent risk assessments for enteroviruses similarly estimate the yearly probabilities of infection near 10^{-4} . However, the inclusion of other emerging pathogens, specifically norovirus, increases this yearly infectious risk by over 2 orders of magnitude. Quantitative microbial risk assessment for biosolids exposure more effectively operates as a tool for analyzing how exposure can be reduced rather than being used to assess “safety”. Such analysis demonstrates that the tradition of monitoring pathogen quality by *Salmonella* spp. and enterovirus content underestimates the infectious risk to the public, and that a rigorous biosolids pathogen treatment process, rather than extending community separation distances, is the most efficient method for reducing pathogen exposure and infectious risk.



INTRODUCTION

In 1987 the U.S. Congress altered section 405 of the Clean Water Act for sewage sludge, requiring the U.S. Environmental Protection Agency (USEPA) to develop an extent program to maximize the beneficial use of sewage sludge while minimizing associated environmental risks. Standards were promulgated in 1992 under the Title 40 Code of Federal Regulations, Part 503, “Standards for the Use or Disposal of Sewage Sludge”.¹ When the U.S. developed and adopted the Part 503 regulations, sewage sludge pathogen content and the potential worker and public exposures to pathogens were poorly understood.² Due to these uncertainties, regulations were based on a framework of expedience—human exposure to pathogens was to be reduced through treatment-based (stabilization) standards and through land application guidelines rather than a risk- or epidemiologically based analysis.³ Sludges were classified as either class A or class B biosolids based on pathogen or fecal indicator concentrations¹ and the sludge treatment used. The class A standard was defined as pathogen free (*Salmonella* spp. below detection levels), whereas class B biosolids could contain pathogens but were expected to pose no public health or environmental risk when guidelines were followed that reduce exposure during and after land application (e.g., reducing vector attractants and restricting site access).¹

Over the last ten years, the scientific basis for the Part 503 regulations has been criticized. These criticisms stem from the lack of biosolids research on pathogen content and aerosol transport, the lack of epidemiological studies, and the growing number of anecdotal health complaints from citizens living near land application sites.^{4–7} As a consequence, 39 of 50 U.S. states have adopted land application rules that are stricter than the federal regulations.⁸ These rules predominantly include more restrictive chemical pollutant and pathogen limits and changes in management practices such as increasing buffer zones between residential areas or drinking water sources and biosolids-applied land.

A USEPA-sponsored National Research Council (NRC) report published in 2002 and a 2003 biosolids summit meeting both described persistent uncertainties associated with the science behind U.S. land application regulations and put forth research agendas to update this science. The overarching NRC technical recommendations included the use of improved risk models to update standards for pathogens and conducting nationwide surveys of pathogen content in sewage sludge. They

Received: February 18, 2011

Accepted: May 26, 2011

Revised: May 18, 2011

Table 1. Log Bacterial and Viral Indicator and Pathogen Concentrations in Stabilized Biosolids per Dry Gram (Values are Means and Standard Deviations and Numbers of Samples are Indicated)

pathogen/indicator quantified	mesophilic anaerobic digestion (US class B) ^a	temperature —phased anaerobic digestion (U.S. class A) ^a	composting (U.S. class A) ^a	references
bacteria				
fecal coliforms (MPN/CFU)	5.8 ± 1.47 US—35, EU—8, Asia—10	2.6 ± 1.85 US—16, EU—2, Asia—1	1.7 ± 1.15 US—18, EU—16, Asia—8	17–19,29,31,70–84
<i>E. coli</i> (MPN/CFU)	5.0 ± 0.59 5.3 ± 0.41 US—4, EU—7, Asia—1	4.1 ± 0.36 NA US—3, EU—1	1.8 ± 1.37 ND US—17, EU—22	15,18,21,29,70,76,85–90
<i>Salmonella</i> spp. (MPN/CFU)	1.6 ± 1.25 NA US—10, EU—7, Asia—11	ND NA US—1, Asia—1	0.64 ± 1.3 2.8 ± 1.86 US—56, EU—15, Asia—8	31,64,71,74,76,79,80,83,85,86,91–95
<i>Salmonella</i> spp. (GU)	NA	NA	0.52 US—8	31
<i>Yersinia</i> spp. (MPN/CFU)	2.6 ± 1.33 US—16	1.8 ± 0.75 US—8	2.5 ± 1.27 US—10	17
<i>Legionella pneumophila</i> (GU)	0.1 ± 0.19 EU—2	NA	ND US—1, EU—1	31,77,85,96
<i>Campylobacter</i> spp. (CFU)	3.9 EU—1	NA	NA	77
<i>Aerobacter butzleri</i> (CFU)	5.3 ± 1.3 5.2 ± 0.66 US—27, EU—13, Asia—10	4.0 ± 0.46 5.2 ± 0.83 US—9, EU—1	3.5 ± 1.65 3.7 ± 1.74 US—18, EU—17, Asia—8	15,18,30,70,74–80,82–84,88,92,93,97
<i>Enterococcus</i> spp. (MPN/CFU)	ND 2.3 ± 1.01 US—16, EU—6	ND 2.4 ± 1.07 US—9	ND ND US—11	17,98
<i>Enterococcus</i> spp. (GU)	2.24 EU—1 1.46 EU—1	NA NA	0.4 ± 0.79 EU—1 0.1 ± 0.33 EU—1	78,92
<i>Staphylococcus aureus</i> (CFU)	6.2 ± 0.55 US—17, EU—5 3.5 ± 2.7	6 ± 0.49 US—8, EU—1	3.8 ± 1.01 US—10, EU—	17–19,56,64,70,88 ^{17,21,84–86,92}
<i>Staphylococcus aureus</i> (GU)	NA US—3, Asia—1 2.3 ± 0.86 US—16	NA NA 2.0 ± 0.85 US—8	12 3.0 ± 1.4 ND EU—2 ND US—10	
<i>Listeria</i> spp. (MPN/CFU)				
<i>L. monocytogenes</i> (MPN/CFU)				
<i>Clostridium</i> spp. (CFU)				
<i>C. perfringens</i> (MPN/CFU)				
<i>C. perfringens</i> (GU)				
viruses				
male-specific coliphages (PFU)	3.4 ± 1.15 US—17, EU—5	2.8 ± 0.81 US—8	0.2 ± 0.43 US—11, EU—5	15,17,19,90,97,99
enterovirus (MPN) enterovirus (GU)	0.9 ± 0.69 4.15 ± 0.5 US—7, EU—5	0.2 ± 0.25 NA US—2	0.4 ± 0.69 1.7 ± 1.11 US—8, EU—3	19,31,38,71,74,82,92,100–104
poliovirus (GU)	3.3 EU—1	NA	NA	40
reovirus (MPN)	1.0 US—1	NA	ND US—1	105
somatic coliphages (PFU)	5.0 ± 1.46 US—1, EU—4	NA	0.6 ± 0.15 EU—5	15,19,84,97
bacteriophages infecting <i>B. fragilis</i> (PFU)	2.6 ± 0.75 EU—4	NA	ND EU—5	15,19,97
adenovirus (GU)	5.1 ± 1.61 US—27, EU—1	3.6 ± 1.55 US—8	3.8 ± 1.54 US—10	16,17,40
norovirus (GU)	±4.5 US—10	NA	NA	16
hepatitis A virus	ND US—10	NA	NA	16

^a Log concentration is based on culturable concentrations (MPN, CFU, PFU) or total qPCR-based concentrations (GU), NA= no analyses reported, ND = not detected.

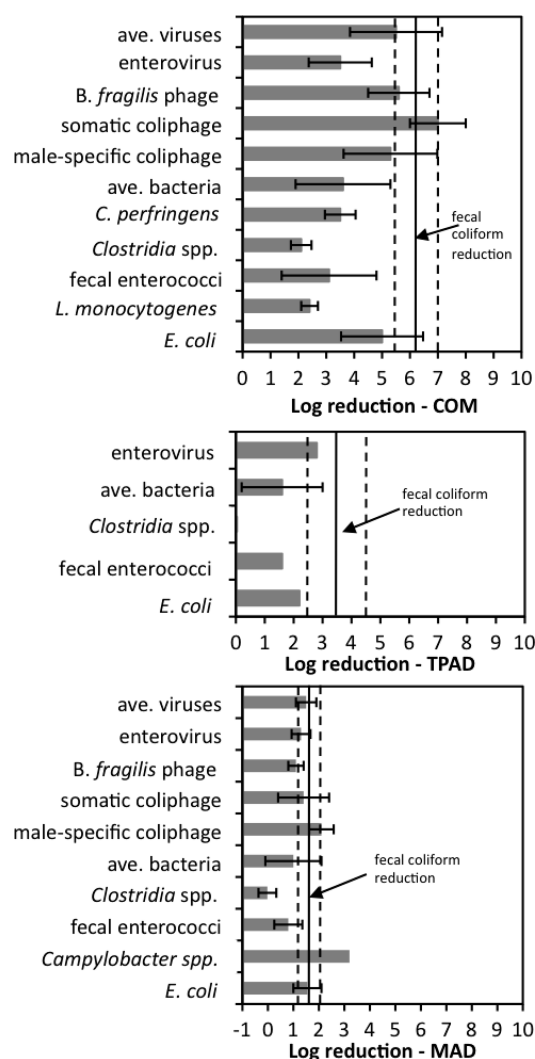


Figure 1. Average log reduction of pathogens and fecal indicators in class A biosolids treated by composting (COM; top)^{17,19,70,85,88,92,100,106} and temperature-phased anaerobic digestion (TPAD; bottom).^{17,18,82,106} Values are composites of multiple log inactivation values measured at full-scale treatment facilities. Error bars represent the standard deviation of inactivation among different studies. For comparison, the log fecal coliform inactivation among the different studies is shown by the solid (mean) and dashed lines (standard deviations).

also suggested development of approaches for human health investigations at land application sites.^{7,9,10} Although only cursory progress has occurred for epidemiology-based human health investigations,^{4,11} research over the last eight years has made significant advances with estimating human exposure and risk to biosolids pathogens. This focus has centered on aerosols as the most significant route of human exposure. Advances include development of theoretically and empirically based microbial aerosol transport models,^{12–14} increased information on pathogen and indicator content in biosolids,^{15–19} and initial attempts to establish residential risks of infection from ingestion and inhalation exposure routes.^{13,20–27}

The goal of this review is to develop a consensus view on the human health impacts associated with land application of class B biosolids. The scope includes residential risk of infection from viral and bacterial pathogens and associated exposure routes, and

excludes chemical compounds, unless direct links to infection can be drawn. All biosolids pathogen and indicator data are analyzed to understand the effectiveness of current standards. Previous risk studies and results from a new risk assessment using the most current pathogen content data and exposure analysis methods are compiled to explore whether a consensus on the magnitude of infectious risk can be formed from these independent studies. Finally, the uncertainties associated with exposure and risk-based approaches are described and tangible recommendations are provided on how risk can be further reduced through sludge pathogen treatment and land application practice modifications.

SLUDGE PATHOGEN CONTENT AND TREATMENT EFFECTIVENESS

A comprehensive literature search was conducted to retrieve and compile concentration information on human pathogens and fecal indicators present in class A and class B biosolids. These compilations were used to judge the effectiveness of sludge treatment on a diversity of pathogens and to populate infectious risk models. A full description of the literature review process and data treatment is presented in the Supporting Information. Briefly, each pathogen or indicator concentration within an individual biosolids study was extracted, log₁₀ transformed, and the means and standard deviations were calculated. Average reductions of pathogens/indicators through sludge treatments were also extracted from literature, log₁₀ transformed, and averaged. In some cases, reductions were also calculated when influent and effluent concentrations were reported for a biosolids treatment, except when effluent values were below detection limits. Only full-scale domestic wastewater treatment plant concentration and reduction results were considered.

Differences in Concentration and Inactivation Effectiveness between Class B and Class A Biosolids Pathogens.

Pathogens and indicators quantified in domestic and international biosolids are shown in Table 1 for common treatment methods including class B mesophilic anaerobic digestion (MAD), class A composting (COM), and class A temperature-phased/thermophilic anaerobic digestion (TPAD). Furthermore, compiled bacterial and viral log culturability/infectivity reductions through MAD, TPAD, and COM treatments are provided in Figure 1 (also see Table S1 for qPCR-based values). Class B biosolids treated by mesophilic anaerobic digestion (MAD) are the most prevalent sludge product in the U.S., accounting for up to 75% of all biosolids applied to land.⁸ Both bacterial and viral pathogens were documented in MAD biosolids at concentrations ranging from 1 to 10⁵ colony forming units (CFU), plaque forming units (PFU), most probable number (MPN), or genomic units (GU) per dry g (Table 1). MAD treatments caused a mean reduction in pathogen/indicator culturability of 1 log, ranging from no reduction in *Clostridia perfringens* to 3.2 logs in *Campylobacter*. Average infectious enterovirus and coliphage reductions were 1.3 logs and 1.75 logs, respectively (Figure 1). Overall, MAD biosolids demonstrated limited inactivation effectiveness at the treatment-plant scale resulting in frequent reports of human bacterial and viral pathogens.

Many biosolids managers are considering upgrades of class B MAD facilities to class A biosolids processes based on either public health concerns or U.S. state and local ordinances that limit a facility's options for reusing class B biosolids. The most common upgrades include either adding a compost process after

MAD treatment/dewatering or adapting MAD processes to a TPAD configuration. TPAD configurations generally include two digesters in series: one operated at thermophilic temperatures (50–55 °C) and one operated at mesophilic temperatures (35–40 °C).²⁸ For this survey, fecal coliform mean concentrations in COM and TPAD-treated sludges were below the 10³ CFU/dry g Part 503 regulatory standard for class A biosolids. Moreover, class A COM and TPAD indicator concentrations were 3–6 logs lower than concentrations in class B MAD biosolids. Pathogens common to class B MAD biosolids were greatly reduced or at nondetectable levels in COM biosolids, including *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Listeria* spp., *Clostridia difficile*, and reovirus (Table 1). Overall, pathogen inactivation in COM studies yielded the highest average inactivation for biosolids treatments analyzed here, with 3.6 logs for bacterial pathogens and fecal indicator bacteria and 5.5 logs for viral pathogens and fecal bacteriophages when MAD inactivation was taken into account (Figure 1).

Limited culture-based bacterial and viral pathogens studies have been conducted for TPAD biosolids, although quantitative PCR has demonstrated the presence of *S. aureus*, *C. difficile*, *L. pneumophila*, and adenovirus genomes (Table 1). The few culture-based studies in existence for TPAD suggest a mean TPAD treatment reduction for culturability/infectivity of 1.6 logs for all fecal indicator bacteria (3.5 logs for fecal coliforms) and 2.8 logs for enteroviruses. These and other more targeted studies^{18,29,30} have expressed concerns about the effectiveness of TPAD processes to inactivate bacterial pathogens and fecal indicators. Studies in thermophilic sludge digesters have shown that fecal coliforms and enterococci were not completely inactivated but instead entered into a viable but not culturable (VBNC) state. The culturability of these indicators could be reactivated during high-speed centrifugation of solids after treatment.

Historically, it was the correlation between fecal coliforms and *Salmonella* spp. that the USEPA used as a basis for monitoring pathogen content by fecal coliform concentrations and setting class A and class B thresholds.³¹ These correlations showed that the probability of *Salmonella* spp. detection was zero when fecal coliform concentrations were less than 10³ MPN/dry g in composted biosolids. Based upon this data, class A biosolids were termed pathogen free when either *Salmonella* spp. were absent or the <10³ fecal coliforms/dry g requirement was met. Class B biosolids are expected to contain pathogens. In general, the compiled data show that plants meet these fecal indicator limits and that ranges in log inactivation generally confirm USEPA expected log reductions of 0.5–3 logs for bacteria and 0.5–2 logs for viruses in class B biosolids. The data further demonstrate that the expectations for enhanced pathogen reduction from class A composting and temperature-phased anaerobic digestion are met.¹

However, class A biosolids are not pathogen free. By compiling culturability-based inactivation in all previous class A biosolids studies, Figure 1 demonstrates that fecal coliform removal or content is a poor indicator of non-*Salmonella* spp. pathogen content in biosolids. Although the 10³ CFU/dry gram standard is met for fecal coliforms in class A biosolids, there were detectable levels (by culturability and qPCR) of pathogens in 8 of the 14 bacterial and viral pathogens described in COM and TPAD literature studies. Moreover, fecal coliform indicators were inactivated more easily during TPAD and COM than all other indicators and pathogens with the exception of somatic coliphages during COM (Figure 1).

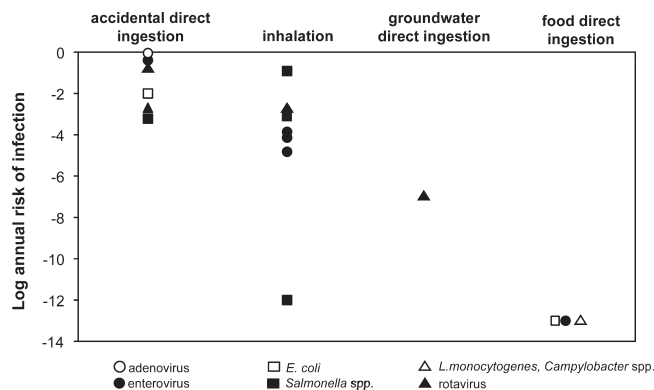


Figure 2. Literature values for annual probability of infection due to biosolids land application for ingestion and inhalation exposure routes. Also see Table S3 for study summaries. Accidental ingestion assumes direct ingestion of 100 mg of biosolids,²⁶ 2 g of biosolids,²⁵ and 50 mg²⁰ of biosolids. For inhalation risks, two land application events were assumed for studies where risk per land application event, rather than annual risk, was estimated.²⁶ Inhalation risks corresponded to a worst case scenario with respect to wind speed and atmospheric stability, and community separation distances included 30-m setbacks^{13,21,26} and 100-m setbacks.²³ Groundwater ingestion risk is determined using a depth of 30 m saturated soil.²⁶ The worst case scenario for food ingestion assumed no decay of pathogens in soil between land application and food harvesting.²⁴

Finally, although qPCR-based methods may not provide reliable log reduction data and were not included in Figure 1, biosolids pathogen content based on PCR is valuable. Clear relationships between qPCR and culturable *Enterococcus* spp. have been observed in biosolids,³⁰ dose–response relationships for norovirus are based on qPCR enumeration,³² and qPCR methods have demonstrated relevance in establishing health outcomes^{33–35} and in estimating specific risk in recreational waters.³⁶ At a very minimum, the presence of these pathogens as indicated by PCR-based methods clearly demonstrates the weakness of assuming that the absence of *Salmonella* spp. indicates the absence of all other pathogens and provides strong rationale for continuing to survey biosolids for the culturable and infective content of a broad diversity of pathogens. Overall, molecular-based studies have pointed to the plausibility of several pathogens in sewage sludge and biosolids including *L. pneumophila*, *C. difficile*, *S. aureus*, methicillin-resistant *S. aureus*, *L. monocytogenes*, pathogenic *Mycobacterium* spp., hepatitis A and E virus, adenovirus, enterovirus, norovirus, parechovirus, coronavirus, and aichi virus.^{16,17,21,37–42} These studies demonstrate that a full and diverse suite of pathogens must be considered when choosing an indicator for sewage sludge treatment. Indeed, with the large potential pathogen diversity in sewage sludge, some level of pathogen monitoring beyond fecal indicators may be required to understand potential risks.

Health Effects and Infectious Risks Associated with Biosolids Application. Epidemiology studies and quantitative microbial risk assessments (QMRAs) have been used to understand the health effects from exposure to biosolids and related pathogens released during land application. Abbreviated notes on the design and results of the two epidemiology studies (Table S2) and the eight QMRA studies (Table S3) that have been conducted thus far are available in the Supporting Information.

Epidemiology Studies. There has been little progress in the last twenty years toward producing epidemiological-based evidence

of biosolids health effects on the public. Indeed, the limited past studies serve only to demonstrate and foster uncertainty about biosolids health effects. The most comprehensive epidemiology study of biosolids exposures to neighboring communities was conducted over 25 years ago and included health surveys and sero-conversion measurements on 163 residents living near land application sites. The study demonstrated no greater incidence of adverse health effects over control groups located away from land application sites.⁴³ These results contrast with a recent mail survey of residents living within one mile of fields permitted for biosolids land application—the 437 resident responses suggested increased incidence of respiratory and gastrointestinal disease over a similarly sized control group living greater than one mile from permitted fields.⁴ Both studies identified potential limitations in their conclusions. The former study had land application rates (2–10 dry metric tons/ha, once per year) that were lower than the norm and the authors recommend caution in extrapolating their outcome to other land application scenarios. The latter study notes the limitations inherent in self-reporting and mail-in surveys—these include recall bias and a tendency to over-report illness when odors are present.^{44,45} Further, epidemiology studies that enroll less than 1000 subjects may not be able to identify statistically relevant health effects unless the risk is very high (greater than 1 in 100 probability of illness). Even then, several more independent studies are needed to form any actionable conclusion.

Additional surveys and questionnaires, while not controlled epidemiology studies, have been used to catalog the self-reported adverse health effects and symptoms of infectious and noninfectious disease of residents near land application sites. Over 350 respondents to these surveys reported health effects within one month of a land application event, including respiratory irritation as well as bacterial, viral, and fungal infections.^{5,46} These survey reports also noted that there were only marginal efforts to track health complaints by the regional USEPA offices and that in almost all cases, anecdotal health complaints were not followed up by a scientific investigation to link health effects to biosolids land application.^{6,7} Follow-up on health complaints has been viewed as a potential method to understand the significance of biosolids on health impacts.^{7,47} Environmental Management System certification, which promotes best practices when land-applying biosolids, has recently been implemented in many U.S. municipalities, however, documented scientific reports of the follow-up of health complaints are not available.

Quantitative Microbial Risk Analyses (QMRA). Potential human health effects due to land application may also be indicated with QMRA. Risk modeling circumvents the epidemiology requirement for a large amount of exposed and unexposed subjects required to observe a low probability of infection. It also allows for consideration of the diverse land application scenarios and meteorological conditions that may impact human exposure. To date, eight independent QMRA studies have reported the biosolids-derived infectious risks to residents living near land application sites (Table S3). Annual infectious risks for different exposure scenarios are summarized in Figure 2 for the different bacterial and viral agents addressed in these studies. Compiling these risks demonstrates that other than accidental ingestion, which the USEPA Part 503 rules address by restricting site access, the largest risk to the public living near biosolids land application operations was from inhalation of aerosols produced during biosolids land application operation (e.g., loading biosolids into application equipment, spreading biosolids onto land, and disk-incorporating

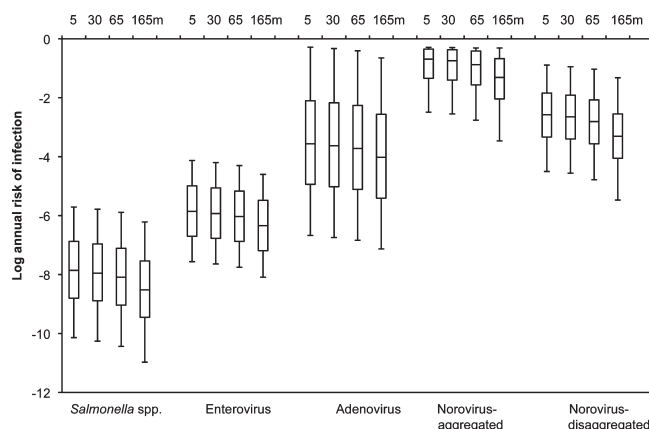


Figure 3. Annual log probability of viral infectious risk associated with aerosols emitted from a biosolids land application event (spreading and diking). Pathogen values are extracted from Table 2. The exposure model¹⁴ considers a worst case scenario of wind velocity = 1.5 m/s and daytime, a highly unstable atmosphere. Top, middle, and bottom horizontal lines in the boxes correspond to 25% percentile, median, and 75% percentile, respectively, and vertical lines represent range. These risks only represent values associated with occurrence (e.g., do not include nondetectable data). Based on the reviewed studies, pathogen occurrence above detection levels in biosolids is 60% for enteroviruses (class B), 88% for adenovirus (class B), and 67% for norovirus (class B).^{16,17}

biosolids into soils). Within these different exposure scenarios, the ranking of risk from highest to lowest was accidental direct ingestion > aerosol inhalation >> contaminated groundwater ingestion > contaminated food ingestion.^{13,21–26} An exception to this general trend may be application of biosolids directly onto Karst or bedrock formations where no attenuation of pathogen transport into groundwater is provided.⁴⁸

These prior risk analyses, however, are potentially very uncertain in magnitude, largely due to the low diversity of bacterial and viral pathogens that have been considered. All human viral and bacterial pathogens can be excreted in urine and feces from which biosolids are derived,⁴⁹ yet only enteroviruses, rotaviruses, and *Salmonella* have been considered in aerosol inhalation risk assessments. Using enteroviruses in risk estimations is expedient due to existing dose–response inhalation models⁵⁰ and the ease in which enteroviruses infectivity can be determined in comparison to other relevant viruses. The most notable agents missing from aerosol risk assessments are adenovirus and norovirus. Infection via inhalation has been demonstrated for each of these agents.^{50–52} Moreover, recent evidence by quantitative PCR suggests that both norovirus and adenovirus^{16,17} are present in significantly higher qPCR concentrations than enteroviruses (Table 1). Norovirus, which is responsible for 90% of non-bacterial outbreaks globally,⁵³ is highly resistant to physical and chemical inactivation,⁵⁴ while adenovirus is a thermally resistant virus that can survive for prolonged periods in the environment.²⁰

■ QUANTITATIVE MICROBIAL RISK ASSESSMENT BASED ON NEW PATHOGEN DATA

To augment previous inhalation risk analyses with pathogen content information and aerosol transport models that have recently become available, we estimated infectious risks for the diversity of pathogens in biosolids aerosols emitted during land application. The criteria for including agents in this QMRA

include (i) existing quantitative pathogen content information in class B biosolids, (ii) documented infection via inhalation or previous consideration in an aerosol risk study, and (iii) a documented human dose–response relationship. We conducted QMRA on *Salmonella* spp. based on its importance in setting biosolids regulations³¹ and its previous consideration in aerosol risk assessments (Figure 2). Viral pathogens that meet all three criteria include adenovirus 4 (representative of adenovirus), coxsackievirus A21 (representative of enteroviruses), and norovirus. This analysis extends aerosol risk estimates to encompass adenovirus and norovirus, and provides another independent assessment of enteroviruses and *Salmonella* spp. aerosol risk.

A full description of the QMRA procedure is presented in the Supporting Information, and a brief description is highlighted here. The inhalable pathogen risk for a land application event (both spreading and dishing operations) was derived from calibrated, first principle-based transport models¹⁴ that employed aerosol reconstruction methods to estimate pathogen exposure (eq 1):

$$\begin{aligned} \text{Dose (pathogen unit)} = & C \left(\frac{\mu\text{g respirable biosolids}}{\text{m}^3} \right) \\ & \times \text{ET(s)} \\ & \times \text{breathing rate} \left(\frac{\text{m}^3}{\text{s}} \right) \\ & \times C_{\text{bulk pathogen}} \left(\frac{\text{pathogen unit}}{\mu\text{g biosolid}} \right) \end{aligned} \quad (1)$$

where a Gaussian plume model with aerosol inactivation was used to estimate downwind biosolids aerosol concentrations under variable emission scenarios and atmospheric stability conditions (C , $\mu\text{g}/\text{m}^3$),¹⁴ an intermittent “puff” exposure time (ET) model was used to determine exposure time based on spreading and dishing equipment movement, wind velocity, and plume dispersion,¹⁴ and aerosol reconstruction was used to convert bulk biosolids pathogen concentrations ($C_{\text{bulk pathogen}}$) to an aerosol pathogen concentration^{55,56} (Figures S1 and S2). Aerosols produced during the spreading of dewatered class B biosolids and dishing of a 16-square-hectare field were considered under a variety of atmospheric stability conditions and wind speeds. Single-hit inhalation dose–response values were based on previous studies in humans for adenovirus and coxsackie virus (representative of enteroviruses).^{9,50,57} For norovirus, which causes gastrointestinal infections and where the airborne route of infection has been observed,^{50–52} the dose–response model for ingestion was used with the assumption that infectious particles captured in the upper respiratory tract are removed by ciliary action and passed into the digestive tract through the pharynx. A value of 50% had been previously used for this fraction in *Salmonella* spp.¹³ Here we use a more conservative range in values of 10% to 50%. The same gastrointestinal infection approach was used for the *Salmonella* spp. dose–response. Concentration inputs into the dose–response model included quantitative PCR values adjusted by the assumption that 1 in 1000 to 1 in 10 000 adenoviruses are infective. These values were retrieved from previous estimates of adenovirus infectivity in contaminated surface waters (1:1000)⁵⁸ and ranged to 1:10 000

to account for infectivity loss in mesophilic anaerobic digesters during class B treatment (Table S1). The range of infective enterovirus and *Salmonella* spp. concentrations was derived from infectivity and culturability values presented in Table 1, and qPCR values were used for norovirus—in accordance with the previously described qPCR-based dose–response relationship.³² Variance in highly uncertain variables including aerosol inactivation, pathogen concentration, and pathogen infectivity was addressed by defining high and low ranges, assuming a uniform or log uniform distribution, and applying Monte Carlo simulations^{20,59,60} to the overall risk calculations.

Distributed inhalation risks of infection for enterovirus, adenovirus, and norovirus with levels of uncertainty are presented in Figure 3. These results indicate that two of the organisms currently used to monitor biosolids pathogen content (*Salmonella* spp. and enterovirus) underestimate the potential infectious risk. At all distances, the highest median and quartile risks were observed for norovirus, which was significantly higher than adenovirus ($p < 0.01$), enterovirus ($p < 0.01$), or *Salmonella* spp. ($p < 0.01$). Median inhalation risks at 30 m from the land application site were near 10^{-1} and 10^{-3} for disaggregated and aggregated norovirus, respectively, and the median risks for adenovirus ($\sim 10^{-4}$) and enteroviruses ($\sim 10^{-6}$) were significantly different ($p < 0.01$). It is currently unclear whether noroviruses are aggregated or disaggregated in the environment—including both provides a more thorough estimate of norovirus risk. The worst-case risk estimates calculated for enteroviruses (10^{-5}) were consistent but on the lower side of independently determined risk estimates for enterovirus (10^{-3} to 10^{-5}). The major sources of uncertainty for the risks presented in Figure 3 in rank order were pathogen concentration > pathogen inactivation > pathogen infectivity.

Figure 3 and Tables 2, S4, and S5 also reveal how atmospheric dispersion, pathogen inactivation, and distance affect inhalation risks. For a given wind velocity, setback distances caused 0.5–1 log median reduction in risk at 165 m from the source, and 1–2 log reductions at 1000 m. For atmospheric conditions, the worst-case scenario (e.g., highest aerosol risk produced at distances less than 500 m) corresponded to the lowest wind-speed simulated (1.5 m/s) and a highly unstable atmosphere (class A stability). This behavior is based on exposure to aerosol “puffs” emitted by a moving tractor and is maximized at low wind speeds due to the longer duration for a puff to pass a stationary receptor.¹⁴

■ PERSISTENT UNCERTAINTIES IN RISK ANALYSIS

Despite the above inclusion of a broader range of pathogens and use of a verified exposure model, questions remain in understanding the inhalation of human pathogens and associated public risk. Much of the uncertainty observed in Figure 3 is inherent. The variability in pathogen concentration among different sludges is real, and although more information on infective concentrations of pathogens in biosolids can define this variability more accurately and precisely, some level of variability cannot be eliminated. It is also recognized that the dose–response information for many pathogens for aerosol or contact exposure is not well described. Although determining infective aerosol dose–response in humans is possible, accurately estimating this value under appropriate conditions may not be feasible given the still unknown diversity of pathogens in sludge, the variable physiological state of pathogens in biosolids and air, different

Table 2. Biosolids Land Application Event PM₁₀ Inhalation Dose (μg)

distance to downwind receptor (m)	dose (μg) at 1.5 m/s, atmos. stab. class A	dose (μg) at 3 m/s, atmos. stab. class B	dose (μg) at 6 m/s, atmos. stab. class C	dose (μg) at 10 m/s, atmos. stab. class C	dose (μg) at 20 m/s, atmos. stab. class C
5	25.3	10.5	3.8	1.4	0.34
30	21.8	8.6	3.2	1.2	0.29
65	17.3	6.9	2.5	0.91	0.23
165	8.8	4.5	1.7	0.59	0.15
500	1.9	1.9	0.84	0.30	0.08
1000	0.54	0.84	0.51	0.18	0.05

susceptibilities in human populations, and barriers to testing in humans. Of particular concern for dose–response is the complex mixture of pathogens, metals, biotoxins, and hazardous organic compounds found in biosolids. Recent in vitro human cell line toxicity experiments with biosolids have demonstrated increased cytotoxicity and inflammatory potential of class B MAD biosolids over agricultural soils and Class A COM biosolids⁶¹ as well as significantly elevated endotoxin, a known inflammatory agent.^{61,62} Elevated lung inflammation has been associated with the increased incidence of infection in murine models.⁶³

Some uncertainty, however, is due to gaps in knowledge that may be better understood with directed research. Significant uncertainties in exposure modeling include a poor understanding of how exposure changes with movement of people and contaminants from outdoor to indoor environments. While advances in the “puff” exposure time model better simulate the land application exposure event,¹⁴ these inhalation doses are still limited to that of an outside, stationary receptor. The use of personal monitoring supported by quantitative biosolids aerosol microbial source-tracking techniques^{12,64} is a more direct approach to better describe the biosolids aerosol dose to residents. A final uncertainty that can be improved upon is the limited data set associated with biosolids risk assessments. Accounting for the uncertainties in risk analysis as well as searching for a weight of evidence requires additional independent assessments. Here, while independent results from different models do result in similar risk estimations near 10^{-4} for enterovirus, it is clear that risk analysis must be expanded to include additional, more relevant pathogens and also that the uncertainty in estimating risk must be evaluated in all risk-based results.

Risk estimates should be used as a means of determining appropriate engineering controls or setting exposure guidelines rather than defining risk as acceptable or not. To make such a judgment, an acceptable risk threshold for biosolids would need to be defined by a regulatory agency. Previously defined risk thresholds from drinking water and recreational water use provide some perspective. Although not a regulation, a traditionally cited risk threshold for the ingestion of drinking water has been a 10^{-4} probability of infection. The basis for this suggestion was that a 10^{-4} probability of infection from *Giardia* spp. per person per year is expected to result in a mean lifetime risk of death from infection of approximately 10^{-5} .⁶⁵ While these microbial risks and cancer are not related, this 10^{-5} level has been cited as reasonable as it is comparable to the commonly used 10^{-4} to 10^{-6} lifetime theoretical risks thresholds for cancer that are used as a basis for estimating maximum contaminant limits for hazardous chemicals in drinking water.⁶⁵ Infectious risk benchmarks have also been determined through epidemiology data. For example, epidemiology studies have indicated that specific fecal indicator densities

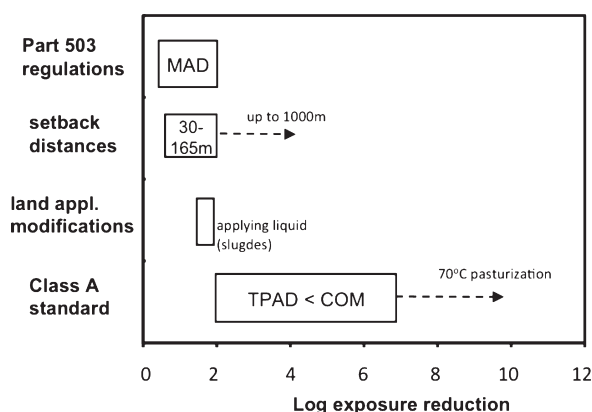


Figure 4. Currently available controls for reducing aerosol exposure associated with biosolids land application. The base case for zero reduction is the land application of raw, dewatered sewage sludge with no setback distance. Reductions can be achieved by MAD treatment (see Table S1), requiring setback distances (Table S4, S5, and S6 and Figure 3), applying liquid rather than dewatered sludges to reduce aerosol generation,^{55,56} or requiring a class A level product (Figure 1).

(126 CFU 100 mL⁻¹ *E. coli* and 35 CFU 100 mL⁻¹ enterococci) would result in highly credible gastrointestinal illness rates between 1 and 2 illnesses per hundred recreation events in waters impacted by treated effluent wastewater,⁶⁶ or 3–4 gastrointestinal illnesses (excludes the need for fever) per recreation event.^{59,67} A definition of acceptable risk in the case of pathogen exposure from biosolids land application may encompass several considerations, including the severity of infections.²⁵ Unlike drinking water regulations, only a small subset of the general population is exposed. Thus if similar risk levels occur between drinking water ingestion and biosolids exposure, the potential amount of infections within the total population will be significantly lower for biosolids. An additional issue is that unlike recreational water regulations, exposure for populations living near biosolids land application sites is not a choice, nor is it easily subject to control (i.e., beach closing). With these caveats in mind, it is crucial to see calculated risk values not as a “safe” or “not safe” binary measure, but as a tool to be used to guide measures for reducing risks.

Methods for Reducing Exposure and Risk to Biosolids Aerosols. These significant uncertainties argue for using biosolids aerosol QMRA models to develop effective approaches for reducing risk. In the U.S., current state and federal biosolids land application regulations purport to limit pathogen exposure to the general public by treating biosolids to reduce pathogen content and/or through modifications to the land application process. Under current federal guidelines (no separation distances and MAD treatment¹), an approximately 1.5 log virus reduction in

exposure exists compared to land-applying untreated sludge with no setback. Our analysis demonstrates that through buffer distances, a class A standard, and modifications in land application practice, reductions in exposure to aerosolized pathogens can be dramatic, reaching 4–9 orders of magnitude if all options were adopted (Figure 4). Although buffer distances set by U.S. states contribute to reductions in exposure, typical separation distances that range from 30 to 165 m only result in a 0.5–1 log reduction in risk (Figure 3 and Table S5). Class A reduction strategies such as composting offer a significantly greater decrease in pathogen exposure and risk (between 2 and 5 logs), and are a more effective method of decreasing infective risk than the use of setbacks—especially in the many locations where setbacks of over 100 m are not feasible.

Beyond separation distances and treatment, biosolids moisture content could also result in decreased pathogen exposure from aerosols. Land-applying dewatered biosolids by side-slinging produces an aerosol emission rate approximately 80 times greater than emission rates observed for liquid sludge spray application.^{56,68} In contrast to dewatered biosolids, liquid sludges need not be stock-piled and loaded on-site into spreaders, thus decreasing the significant aerosols that are generated during spreader loading.²¹ Increased moisture also has benefits for reducing aerosols during disk incorporation. Adding dewatered biosolids to dry Arizona soils increased the soil moisture content from 4.8% to 8% and reduced the total PM₁₀ emissions produced during disking by at least three times.⁵⁵ Since dewatered sludge transport is more cost-effective than liquid sludge, this drop in exposure must be balanced with the costs of hauling liquid versus dewatered sludge.

CONCLUSIONS AND THE PATH FORWARD

The sustainability of modern domestic wastewater treatment is dependent upon the safety of biosolids reuse or disposal. To provide new and important insights into biosolids land application and human health, this review conducted a literature survey on pathogen content and inactivation, compiled and analyzed the results of previous risk studies, and produced new risk estimates using the most up-to-date pathogen content information. Pathogen survey results clearly demonstrate that fecal coliform indicator concentration and associated class A treatment or monitoring requirements do not confirm that class A biosolids are pathogen free, nor is fecal coliform inactivation a conservative measure of pathogen inactivation. The comparison of available quantitative risk studies suggests that, other than accidental ingestions, which the Part 503 regulations to prevent site access are aimed at reducing, aerosols were identified to be the most important route of human exposure to infectious agents. Although there is a consensus among independent risk assessments on the probability of aerosol infection from enteroviruses, inclusion of adenovirus and especially norovirus in risk estimates clearly demonstrates that previous and current risk values for enteroviruses and *Salmonella* spp. are an underestimate of the total infective risk of pathogens contained in biosolids. Moreover, the consideration of pathogens (such as norovirus) in risk analysis that were not considered during the original analysis for Part 503 rule demonstrates that previous standards based on *Salmonella* spp. and enterovirus will not achieve the level of protection intended in these regulations. Whereas inclusion of these new pathogens improves our understanding of risk, they are still only a better set of indicators, and their absence can never

ensure the complete lack of infectious risk. Finally, there is large uncertainty in these aerosol risk values, due primarily to limitations in current exposure models, pathogen content, and dose–response information. The lack of a clearly defined acceptable risk threshold for residents living near biosolids land application sites precludes the use of risk as a definitive measure of safety. Quantitative microbial risk assessment for biosolids more effectively operates as a tool for analyzing how exposure can be reduced. Such analysis here demonstrates that a rigorous biosolids pathogen treatment process, rather than extending community separation distances, is a more efficient method for reducing pathogen exposure and infectious risk.

The USEPA originally promulgated biosolids rules based on expedience—reducing pathogen exposure through treatment by the most direct and least-costly approach in view of the uncertainty associated with taking a more risk-based precautionary approach. The USEPA recently put forward its intention to reevaluate the Part 503 biosolids pathogen rule based on a risk analysis framework.⁶⁹ This is a valid path forward only if efforts are made to reduce the uncertainties in risk estimations, an acceptable level of risk or targeted level of reduction for risk is defined, agents such as norovirus and adenovirus are included, and results are applied to interpret and improve the original treatment technologies and regulations put forward in Part 503.

ASSOCIATED CONTENT

S Supporting Information. Additional tables that detail the log inactivation of pathogens and indicators during biosolids treatment (Table S1), and summarize previous biosolids epidemiology (Table S2) and risk studies (Table S3); a detailed QMRA methods section and QMRA description including data on real-time measurements of aerosols emitted from land application events (Figure S1) and information to demonstrate the basis of aerosol reconstruction (Figure S2); for variable downwind setback distances, the pathogen inhalation doses (Table S4) and the probability of infection (Table S5) that were estimated herein. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Phone: (203) 432-4385; fax: (203) 432-4387; e-mail: Jordan.Peccia@yale.edu

ACKNOWLEDGMENT

This research was funded by a U.S. National Science Foundation CAREER grant (BES 0650379) awarded to J.P. K.B. was supported by the STAR Fellowship Assistance Agreement FP917115 awarded by the USEPA. This manuscript has not been formally reviewed by the EPA. The views expressed in this article are solely those of the authors, and the USEPA does not endorse any products or commercial services mentioned in this article.

REFERENCES

- (1) USEPA. *Land Application of Sewage Sludge: A Guide for Land Appliers on the Requirements of the Federal Standard for the Use or Disposal of Sewage Sludge*, 40 CFR Part 503; EPA/831-B-93-002b; Washington, DC, 1994.

- (2) Vesilind, A. Sludge disposal: ethics and expediency. *Water Sci. Technol.* **2000**, 42, 1–5.
- (3) USEPA. *Pathogen Risk Assessment for Land Application in Municipal Sludge. Vol 1: Methodology and Computer Model*; PB90-17190; Washington, DC, 1989.
- (4) Khuder, S.; Milz, S. A.; Bisesi, M.; Vincent, R.; McNulty, W.; Czajkowski, K. Health survey of residents living near farm fields permitted to receive biosolids. *Arch. Environ. Occup. Health* **2007**, 62, 5–11.
- (5) Harrison, E.; Oaks, S. R. Investigation of alleged health incidents associated with land application of sewage sludges. *New Solutions* **2002**, 12, 387–408.
- (6) Lewis, D. L.; Gattie, D. K. Pathogen risks from applying sewage sludge to land. *Environ. Sci. Technol.* **2002**, 36, 286A–293A.
- (7) National Research Council. *Biosolids Applied to Land- Advancing Standards and Practices*; The National Academies Press: Washington, DC, 2002.
- (8) Beecher, N.; Crawford, K.; Goldstein, N.; Lono-Batura, M.; Dziezyk, E. A national biosolids regulation, quality, end use, and disposal survey; <http://www.nebiosolids.org/uploads/pdf/NtlBiosolidsReport-20July07.pdf>; Northeast Biosolids and Residuals Association: Tamworth, NH, 2007.
- (9) Crabtree, K. D.; Gerba, C. P.; Rose, J. B.; Haas, C. N. Waterborne adenovirus: a risk assessment. *Water Sci. Technol.* **1997**, 35, 1–6.
- (10) O'Connor, G. A.; Elliot, H. A.; Basta, N. T.; Bastian, R. K.; Pierzynski, G. M.; Sims, R. C.; Smith, J. E., Jr. Sustainable land application: An overview. *J. Environ. Qual.* **2005**, 34, 7–17.
- (11) Viau, E.; Levi-Schaffer, F.; Peccia, J. Respiratory toxicity and inflammatory response in human bronchial epithelial cells exposed to biosolids, animal manure, and agricultural soil particulate matter. *Environ. Sci. Technol.* **2010**, 44, 3142–3148.
- (12) Baertsch, C.; Paez-Rubio, T.; Viau, E.; Peccia, J. Aerosol source tracking of anaerobically digested sewage sludge released during land application. *Appl. Environ. Microbiol.* **2007**, 73, 4522–4531.
- (13) Brooks, J. P.; Tanner, B. D.; Gerba, C.; Haas, C.; Pepper, I. Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically derived transport model. *J. Appl. Microbiol.* **2005**, 98, 397–405.
- (14) Low, S. Y.; Paez-Rubio, T.; Baertsch, C.; Kucharski, M.; Peccia, J. Off-Site Exposure to Respirable Aerosols Produced during the Disk-Incorporation of Class B Biosolids. *J. Environ. Eng.* **2007**, 133, 987–994.
- (15) Mandilara, G. D.; Smeti, E. M.; Mavridou, A. T.; Lambiri, M. P.; Vatopoulos, A.; Riga, F. P. Correlation between bacterial indicators and bacteriophages in sewage and sludge. *FEMS Microbiol. Lett.* **2006**, 263, 119–126.
- (16) Wong, K.; Onan, B. M.; Xagoraki, I. Quantification of Enteric Viruses, Pathogen Indicators, and Salmonella Bacteria in Class B Anaerobically Digested Biosolids by Culture and Molecular Methods. *Appl. Environ. Microbiol.* **2010**, 76, 6441–6448.
- (17) Viau, E.; Peccia, J. A survey of wastewater indicators and human pathogen genomes in biosolids produced by class A and class B stabilization treatments. *Appl. Environ. Microbiol.* **2009**, 75, 164–174.
- (18) Sahlstrom, L.; Aspan, A.; Bagge, E.; Danielsson-Tham, M.-L.; Albiñ, A. Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. *Water Res.* **2004**, 38, 1989–1994.
- (19) Guzmán, C.; Jofre, J.; Montemayor, M.; Lucena, F. Occurrence and levels of indicators and selected pathogens in different sludges and biosolids. *J. Appl. Microbiol.* **2007**, 103, 2420–2429.
- (20) Eisenberg, J. N.; Seto, E. Y. W.; Olivieri, A. W.; Spear, R. C. Quantifying Water Pathogen Risk in an Epidemiological Framework. *Risk Anal.* **1996**, 16, 549–563.
- (21) Brooks, J. R.; Tanner, B. D.; Josephson, K. L.; Gerba, C.; Haas, C. N.; Pepper, I. A national survey on the residential impact of biological aerosols from the land application of biosolids. *J. Appl. Microbiol.* **2005**, 99, 310–322.
- (22) Brooks, J. P.; Gerba, C. P.; Pepper, I. Biological Aerosol Emission, Fate, and Transport from Municipal and Animal Wastes. *J. Residuals Sci. Technol.* **2004**, 1, 16–28.
- (23) Dowd, S. E.; Gerba, C. P.; Pepper, I. L.; Pillai, S. D. Bioaerosol transport modeling and risk assessment in relation to biosolid placement. *J. Environ. Qual.* **2000**, 29, 343–348.
- (24) Gale, P. Land application of treated sewage sludge: quantifying pathogen risks from consumption of crops. *J. Appl. Microbiol.* **2005**, 98, 380–396.
- (25) Westrell, T.; Schonning, C.; Stenstrom, T. A.; Ashbolt, N. J. QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis and critical control points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Sci. Technol.* **2004**, 50, 23–30.
- (26) Eisenberg, J. N.; Moore, K.; Soller, J. A.; Eisenberg, D.; Colford, J. M. J. Microbial risk assessment framework for exposure to amended sludge projects. *Environ. Health Perspect.* **2008**, 116, 727–733.
- (27) Eisenberg, J. N. S.; Soller, J. A.; Scott, J.; Eisenberg, D. M.; Colford, J. M., Jr. A dynamic model to assess microbial health risks associated with beneficial uses of biosolids. *Risk Anal.* **2004**, 24, 221–236.
- (28) Godfree, A.; Farrell, J. Processes for managing pathogens. *J. Environ. Qual.* **2005**, 34, 105–113.
- (29) Higgins, M. J.; Yen-Chih, C.; Murthy, S. N.; Hendrickson, D.; Farrell, J.; Schafer, P. L. Reactivation and growth of non-culturable indicator bacteria in anaerobically digested biosolids after centrifuge dewatering. *Water Res.* **2007**, 41, 665–673.
- (30) Viau, E.; Peccia, J. Evaluation of the enterococci indicator in biosolids using culture-based and quantitative PCR assays. *Water Res.* **2009**, 43, 4878–4887.
- (31) Yanko, W. A. *Occurrence of Pathogens in Distribution and Marketing Municipal Sludges*; EPA/600/S1-87/014; USEPA: Research Triangle, NC, 1988.
- (32) Teunis, P. F.; Moe, C. L.; Liu, P.; Miller, S. E.; Lindesmith, L.; Baric, R. S.; Le Pendu, J.; Calderon, R. L. Norwalk virus: how infectious is it? *J. Med. Virol.* **2008**, 80, 1468–76.
- (33) Colford, J. M. J.; Wade, T. J.; Schiff, K. C.; Wright, C. C.; Griffith, J. F.; Sandhu, S. K.; Burns, S.; Sobsey, M.; Lovelace, G.; Weisberg, S. B. Water Quality Indicators and the Risk of Illness at Beaches With Nonpoint Sources of Fecal Contamination. *Epidemiology* **2007**, 18, 27–35.
- (34) Wade, T. J.; Calderon, R. L.; Brenner, K. P.; Sams, E.; Beach, M.; Haugland, R.; Wymer, L.; Dufour, A. P. High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. *Epidemiology* **2008**, 19, 375–83.
- (35) Wade, T. J.; Pai, N.; Eisenberg, J. N. S.; Colford, J. M., Jr. Do U. S. Environmental Protection Agency Water Quality Guidelines for Recreational Waters Prevent Gastrointestinal Illness? A Systematic Review and Meta-analysis. *Environ. Health Perspect.* **2003**, 111, 102–109.
- (36) Soller, J. A.; Bartrand, T.; Ashbolt, N. J.; Ravenscroft, J. E.; Wade, T. J. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Res.* **2010**, 44, 4736–4747.
- (37) Borjesson, S.; Matussek, A.; Melin, S.; Lofgren, S.; Lindgren, P. E. Methicillin resistant *Staphylococcus aureus* (MRSA) in municipal wastewater: an uncharted threat? *J. Appl. Microbiol.* **2010**, 108, 1244–1251.
- (38) Straub, T. M.; Pepper, I. L.; Gerba, C. P. Detection of naturally occurring enteroviruses and hepatitis A virus in undigested and anaerobically digested sludge using the polymerase chain reaction. *Can. J. Microbiol.* **1994**, 40, 884–888.
- (39) Kerouanton, A.; Roche, S. M.; Marault, M.; Velge, P.; Pourcher, A. M.; Brisabois, A.; Federighi, M.; Garrec, N. Characterization of isolates of *Listeria monocytogenes* from sludge using pulsed-field gel electrophoresis and virulence assays. *J. Appl. Microbiol.* **2010**, 108, 1380–1388.
- (40) Bofill-Mas, S.; Albinana-Gimenez, N.; Clemente-Casares, P.; Hundesa, A.; Rodriguez-Manzano, J.; Allard, A.; Calvo, M.; Girones, R. Quantification and Stability of Human Adenoviruses and Polyomavirus JCPyV in Wastewater Matrices. *Appl. Environ. Microbiol.* **2006**, 72, 7894–7896.
- (41) Bibby, K.; Viau, E.; Peccia, J. Pyrosequencing of the 16S rRNA gene to reveal bacterial pathogen diversity in biosolids. *Water Res.* **2010**, 44, 4252–4260.

- (42) Bibby, K.; Viau, E.; Peccia, J. Viral metagenome analysis to guide human pathogen monitoring in environmental samples. *Lett. Appl. Microbiol.* **2011**, *52*, 386–392.
- (43) Dorn, C.; Reddy, C.; Lamphere, D.; Gaeuman, J.; Lanese, R. Municipal Sewage Sludge Application on Ohio Farms: Health Effects. *Environ. Res.* **1985**, *38*, 332–359.
- (44) Schiffman, S. S.; Saltely-Miller, E. A.; Suggs, M. S.; Graham, B. G. The effect of environmental odors emanating from commercial swine operation on the mood of nearby residents. *Brain Res. Bull.* **1995**, *37*, 369–375.
- (45) Shusterman, D. J.; Liscomb, J.; Neutra, R.; Satin, K. Sympom prevalence and odor-worry interaction near hazardous waste sites. *Environ. Health Perspect.* **1991**, *94*, 25–30.
- (46) Lewis, D. L.; Gattie, D. K.; Novak, M. E.; Sanchez, S.; Pumphrey, C., Interactions of pathogens and irritant chemicals in land-applied sewage sludges (biosolids). *BMC Pub. Health* **2002**, *2*, (11).
- (47) Goldstein, N.; Striano, E. Summit Identifies Biosolids Research Need. *Biocycle* **2003**, *44*, 67.
- (48) Eisenberg, J. N. S.; Moore, K.; Colford, J. M.; Soller, J. A.; Eisenberg, D. M. *Application of a Dynamic Model to Assess Microbial Health Risks Associated with Beneficial Uses of Biosolids*; 98-REM-1a; WERF, Alexandria, VA, 2006.
- (49) Sinclair, R.; Boone, S. A.; Greenberg, D.; Keim, P.; Gerba, C. P. Persistence of category A select agents in the environment. *Appl. Environ. Microbiol.* **2008**, *74*, 555–563.
- (50) Knight, V. Airborne Transmission and Pulmonary Deposition of Respiratory Viruses. In *Fourth International Symposium on Aerobiology*, Oosthoek, Utrecht, The Netherlands, 1973; Windler, K. C., Ed.; Oosthoek: Utrecht, The Netherlands, 1973; pp 175–182.
- (51) Chadwick, P.; McCann, R. Transmission of a small round structured virus by vomiting during a hospital outbreak of gastroenteritis. *J. Hosp. Infect.* **1994**, *26*, 251–259.
- (52) Chadwick, P.; Walker, M.; Rees, A. Airborne transmission of a small round structured virus. *Lancet* **1994**, *343*, 171.
- (53) Lindesmith, L.; Moe, C.; Marionneau, S.; Ruvoen, N.; Jiang, X.; Lindblad, L.; Stewart, P.; LePend, J.; Baric, R. Human susceptibility and resistance to Norwalk virus infection. *Nat. Med.* **2003**, *9*, 548–553.
- (54) Dolin, R. Noroviruses-Challenges to control. *N. Engl. J. Med.* **2007**, *357*, 1072–1073.
- (55) Paez-Rubio, T.; Xin, H.; Anderson, J.; Peccia, J. Particulate matter composition and emission rates from the disk incorporation of class B biosolids into soil. *Atmos. Environ.* **2006**, *40*, 7034–7045.
- (56) Paez-Rubio, T.; Ramarui, A.; Sommer, J.; Xin, H.; Anderson, J.; Peccia, J. Emission rates and characterization of aerosols produced during the spreading of dewatered class B biosolids. *Environ. Sci. Technol.* **2007**, *41*, 3537–3544.
- (57) Couch, R. B.; Douglas, R. G.; Lindgren, K. M.; Gerone, P. J.; Knight, V. Airborne Transmission of Respiratory Infection With Cocksackievirus A Type 21. *Am. J. Epidemiol.* **1970**, *91*, 78–86.
- (58) Jothikumar, N.; Cromeans, T. L.; Hill, V. R.; Lu, X.; Sobsey, M. D.; Erdman, D. D. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. *Appl. Environ. Microbiol.* **2005**, *71*, 3131–3136.
- (59) Soller, J. A.; Schoen, M. E.; Bartrand, T.; Ravenscroft, J. E.; Ashbolt, N. J. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Res.* **2010**, *44*, 4674–4691.
- (60) Eisenberg, J. N. S.; Seto, E. Y. W.; Colford, J. M., Jr.; Olivieri, A.; Spear, R. C. An Analysis of the Milwaukee Cryptosporidiosis Outbreak Based on a Dynamic Model of the Infection Process. *Epidemiology* **1998**, *9*, 255–263.
- (61) Viau, E. Human Pathogenic and Lung Inflammatory Aerosol Exposures Associated with the Land Application of Biosolids. Ph.D. Dissertation, Yale University, New Haven, CT, 2009.
- (62) Brooks, J. P.; Tanner, B. D.; Gerba, C. P.; Pepper, I. L. The measurement of aerosolized endotoxin from land application of Class B biosolids in southeast Arizona. *Can. J. Microbiol.* **2006**, *52*, 150–156.
- (63) Harrod, K.; Jaramillo, R.; Rosenberger, C.; Wang, S.; Berger, J.; McDonald, J.; Reed, M. Increased susceptibility to RSV infection by exposure to inhaled diesel engine emissions. *Am. J. Respir. Cell Mol. Biol.* **2003**, *28*, 451–463.
- (64) Dowd, S.; Widmer, K.; Pillai, S. D. Thermotolerant Clostridia as an airborne pathogen indicator during land application of biosolids. *J. Environ. Qual.* **1997**, *26*, 194–199.
- (65) Macier, B.; Regli, S. *Use of Microbial Risk Assessment in Setting U.S. Drinking Water Standards*; EPA 814/S-92-001; Washington, DC, 1992.
- (66) USEPA. *Ambient Water Quality Criteria for Bacteria*; EPA440/5-84-002; Washington, DC, 1986.
- (67) Wade, T. J.; Calderon, R. L.; Brenner, K. P.; Sams, E.; Beach, M.; Haugland, R.; Wymer, L.; Dufour, A. P. High sensitivity of children to swimming-associated gastrointestinal illness: Results using a rapid assay of recreational water quality. *Epidemiology* **2008**, *19*, 375–383.
- (68) Tanner, B. D.; Brooks, J. P.; Haas, C. N.; Gerba, C. P.; Pepper, I. L. Bioaerosol Emission Rate and Plume Characteristics during Land Application of Liquid Class B Biosolids. *Environ. Sci. Technol.* **2005**, *39*, 1584–1590.
- (69) USEPA. *Problem Formulation of Human Health Risk Assessments of Pathogens in Land-Applied biosolids*; EPA/600/R-08/035A; Washington DC, 2010.
- (70) Brianescio, R.; Coccia, A. M.; Chiaretti, G.; Libera, S. D.; Semproni, M.; Bonadonna, L. Assessment of microbiological and parasitological quality of composted wastes: health implications and hygienic measures. *Waste Manage. Res.* **2008**, *26*, 196–202.
- (71) Iranpour, R.; Cox, H. H. J. Evaluation of thermophilic anaerobic digestion processes for full-scale class A biosolids disinfection at Hyperion Treatment Plant. *Biotechnol. Bioeng.* **2007**, *97*, 19–39.
- (72) Qi, Y.; Dentel, S. K.; Herson, D. S. Increases in fecal coliform bacteria resulting from centrifugal dewatering of digested biosolids. *Water Res.* **2007**, *41*, 571–580.
- (73) Zabranska, J.; Dohanyos, M.; Jenicek, P.; Ruzickova, H.; Vranova, A. Efficiency of autothermal thermophilic aerobic digestion and thermophilic anaerobic digestion of municipal wastewater sludge in removing Salmonella spp. and indicator bacteria. *Water Sci. Technol.* **2003**, *47*, 151–156.
- (74) Zierzghi, H.; Gerba, C. P.; Brooks, J. P.; Pepper, I. L. Long-term effects of land application of class B biosolids on the soil microbial populations, pathogens, and activity. *J. Environ. Qual.* **2010**, *39*, 402–408.
- (75) Bonjoch, X.; Blanch, A. R. Resistance of faecal coliforms and enterococci populations in sludge and biosolids to different hygienisation treatments. *Microbial Ecol.* **2009**, *57*, 478–483.
- (76) Christensen, K. K.; Carlsbaek, M.; Kron, E. Strategies for evaluating the sanitary quality of composting. *J. Appl. Microbiol.* **2002**, *92*, 1143–1158.
- (77) Stampi, S.; De Luca, G.; Varoli, O.; Zanetti, F. Occurrence, removal and seasonal variation of thermophilic campylobacters and arcobacter in sewage sludge. *Zentralbl. Hyg. Umweltmed.* **1999**, *202*, 19–27.
- (78) de Luca, G.; Zanetti, F.; Fateh-Moghadam, P.; Stampi, S. Occurrence of *Listeria monocytogenes* in sewage sludge. *Zentralbl. Hyg. Umweltmed.* **1998**, *201*, 269–277.
- (79) Watanabe, T.; Kitamura, T.; Ochi, S.; Ozaki, M. Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Water Sci. Technol.* **1997**, *36*, 25–32.
- (80) Pillai, S. D.; Widmer, K.; Dowd, S.; Riche, S. C. Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. *Appl. Environ. Microbiol.* **1996**, *62*, 296–299.
- (81) Skanavis, C.; Yanko, W. A. Evaluation of composted sewage sludge based on soil amendments for potential risks of salmonellosis. *J. Environ. Health* **1994**, *56*, 19–24.
- (82) Berg, G.; Berman, D. Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. *Appl. Environ. Microbiol.* **1980**, *39*, 361–368.
- (83) Dahab, M.; Surampalli, R. Effects of aerobic and anaerobic digestion systems on pathogen and pathogen indicator reduction in municipal sludge. *Water Sci. Technol.* **2002**, *46*, 181–187.

- (84) Chauret, C.; Springthorpe, S.; Sattar, S. Fate of Cryptosporidium oocysts, Giardia Cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Can. J. Microbiol.* **1999**, *45*, 257–262.
- (85) Wery, N.; Lhoutellier, C.; Ducray, F.; Delgenes, J.-P.; Godon, J.-J. Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. *Water Res.* **2008**, *42*, 53–62.
- (86) Eamens, G. J.; Waldron, A. M.; Nicholls, P. J. Survival of pathogenic and indicator bacteria in biosolids applied to agricultural land. *Aust. J. Soil Res.* **2006**, *44*, 647–659.
- (87) Chen, Y. C.; Higgins, M. J.; Maas, N. A.; Murthy, S. N. DNA extraction and *Escherichia coli* quantification of anaerobically digested biosolids using the competitive touchdown PCR method. *Water Res.* **2006**, *40*, 3037–3044.
- (88) Gantzer, C.; Gaspard, P.; Galvez, L.; Huyard, A.; Dumouthier, N.; Schwartzbrod, J. Monitoring of bacterial and parasitological contamination during various treatment of sludge. *Water Res.* **2001**, *35* (16), 3763–3770.
- (89) Soares, H. M.; Cardenas, B. Evaluating pathogen regrowth in biosolids compost. *Biocycle* **1995**, *36*, 70–74.
- (90) Lang, N. L.; Bellett-Travers, M. D.; Smith, S. R. Field investigations on the survival of *Escherichia coli* and presence of other enteric micro-organisms in biosolids-amended agricultural soil. *J. Appl. Microbiol.* **2007**, *103*, 1868–1882.
- (91) Novinscak, A.; Surette, C.; Filion, M. Quantification of *Salmonella* spp. in composted biosolids using a TaqMan qPCR assay. *J. Microbiol. Methods* **2007**, *70*, 119–126.
- (92) Pourcher, A. M.; Morand, P.; Picard-Bonnaud, F.; Billaudel, S.; Monpoeho, S.; Federighi, M.; Ferre, V.; Moguedet, G. Decrease of enteric micro-organisms from rural sewage sludge during their composting in straw mixture. *J. Appl. Microbiol.* **2005**, *99*, 528–539.
- (93) Jepsen, S.-E.; Krause, M.; Gruttner, H. Reduction of fecal streptococcus and *Salmonella* by selected treatment methods for sludge and organic waste. *Water Sci. Technol.* **1997**, *36*, 203–210.
- (94) Hay, J. C. Pathogen destruction and biosolids composting. *Biocycle* **1996**, *37*, 67–76.
- (95) Hussong, D.; Burge, W. D.; Enkiri, N. K. Occurrence, growth, and suppression of salmonellae in composted sewage sludge. *Appl. Environ. Microbiol.* **1985**, *50*, 887–893.
- (96) Jones, K.; Betaieb, M.; Telford, D. R. Seasonal variation of thermophilic campylobacters in sewage sludge. *J. Appl. Bacteriol.* **1990**, *69*, 185–189.
- (97) Lasobras, J.; Dellunde, J.; Jofre, J.; Lucena, F. Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges. *J. Appl. Microbiol.* **1999**, *86*, 723–729.
- (98) Rusin, P.; Maxwell, S.; Brooks, J.; Gerba, C.; Pepper, I. Evidence for the absence of *Staphylococcus aureus* in land applied biosolids. *Environ. Sci. Technol.* **2003**, *18*, 4027–4030.
- (99) Mignotte-Cadiegues, B.; Gantzer, C.; Schwartzbrod, J. Evaluation of bacteriophages during the treatment of sludge. *Water Sci. Technol.* **2002**, *46*, 189–194.
- (100) Monpoeho, S.; Maul, A.; Bonnin, C.; Patria, L.; Ranarijaona, S.; Billaudel, S.; Ferre, V. Clearance of human-pathogenic viruses from sludge: Study of four stabilization processes by real-time reverse transcription-PCR and cell culture. *Appl. Environ. Microbiol.* **2004**, *70*, 5434–5440.
- (101) Schwartzbrod, L.; Mathieu, C. Virus recovery from wastewater treatment plant sludges. *Water Res.* **1986**, *20*, 1011–1013.
- (102) Goyal, S. M.; Schaub, S. A.; Wellings, F. M.; Berman, D.; Glass, J. S.; Hurst, C. J.; Brashear, D. A.; Sorber, C. A.; Moore, B. E.; Bitton, G.; Gibbs, P. H.; Farrah, S. R. Round robin investigation of methods for recovering human enteric viruses from sludge. *Appl. Environ. Microbiol.* **1984**, *48*, 531–538.
- (103) Goddard, M. R.; Bates, J.; Butler, M. Recovery of indigenous enteroviruses from raw and digested sewage sludges. *Appl. Environ. Microbiol.* **1981**, *42*, 1023–1028.
- (104) Soares, A. C.; Straub, T. M.; Pepper, I. L.; Gerba, C. P. Effect of anaerobic digestion on the occurrence of enteroviruses and *Giardia* cysts in sewage sludge. *J. Environ. Sci. Health, Part A* **1994**, *29*, 1887–1897.
- (105) Gallagher, E. M.; Margolin, A. B. Development of an integrated cell-culture-real-time RT-PCR assay for detection of reovirus in biosolids. *J. Virol. Methods* **2007**, *139*, 195–202.
- (106) Watanabe, H.; Kitamura, T.; Ochi, S.; Ozaki, M. Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Water Sci. Technol.* **1997**, *36*, 25–32.