

# **Toward a Consensus View on the Infectious Risks Associated with Land Applying Sewage Sludge**

## **SUPPORTING INFORMATION**

5 tables  
2 figures  
23 pages

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## **PATHOGEN LITERATURE SURVEY**

This literature search utilized the following computerized databases: Web of Science (<http://isiknowledge.com>), MEDLINE (<http://www.ncbi.nlm.nih.gov>), Google Scholar (<http://www.google.com>), SCOPUS (<http://www.scopus.com>), Compendex (<http://www.engineeringvillage2.org>), the UMI/ProQuest Dissertation Database (<http://wwwlib.umi.com/dissertations/gateway>), and the U.S. government science information database (<http://www.science.gov>). Literature was sought in the following areas: biosolids pathogen measurement and treatment method effectiveness, field measurement and transport modeling of aerosols during land application of biosolids, biosolids and health effects, and quantitative microbial risk assessment in biosolids. Searches included combinations of the following key terms: biosolids, sludge, sewage sludge, compost, anaerobic digestion, land application, agriculture, pathogen, virus, aerosol, exposure, risk, quantitative microbial risk assessment, health effects, source emission rate, atmospheric transport, Gaussian, quantitative PCR, and inactivation. Recovered manuscripts were reviewed for relevancy and additional references were extracted from bibliographies.

To assess treatment effectiveness and populate risk models, the review first investigated bacterial and viral pathogens and indicator content in biosolids originating from municipal wastewater treatment. Studies were included if they reported at least one quantitative culture or quantitative PCR (qPCR) pathogen or fecal indicator measurement for the following biosolids treatment methods: mesophilic anaerobic digestion (MAD), temperature-phased/thermophilic anaerobic digestion (TPAD), and/or composting (COM). These methods correspond to the most common treatment techniques for class B (MAD) and class A (TPAD, COM) biosolids <sup>(1)</sup>. Pathogen studies were excluded if the sludge was from industrial (e.g. pulp mill), drinking water,

household, or animal sources, or if uncommon modifications to the typical treatment process were made (e.g. vermiculture composting). Parasitic pathogen data (e.g. *Giardia*) were not included.

**Pathogen data abstraction.** All pathogen data were converted to a per dry gram solids basis. If solids content was not reported, the average liquid or dewatered solids content for that treatment or dewatering method was used. For each pathogen, values were extracted from studies,  $\log_{10}$  transformed, and the means and standard deviations calculated <sup>(2, 3)</sup>. To determine the average reduction of indicators or pathogens through a treatment method, all reported reduction values were also extracted from literature, log transformed, and standard deviations calculated. Full inactivation values are presented in **Table S1**. Reported COM inactivation values reflect inactivation only from composting. TPAD inactivation values include the overall inactivation for both phases. Only pathogen concentration and reduction results from full-scale wastewater treatment plants were considered.

**TABLE S1. Log Inactivation of Indicators and Pathogens Through Biosolids Treatment.**

indicator or pathogen inactivated during treatment	mesophilic anaerobic digestion (class B) <sup>a,b</sup>	temperature – phased anaerobic digestion (class A) <sup>a,b,e</sup>	composting (class A) <sup>a,b,f</sup>	references
fecal coliforms	1.5 ± 0.45	3.5 ± 1.03	4.6 ± 0.60	(4-10)
<i>Escherichia coli</i>	1.5 ± 0.55	2.2	3.5 ± 1.37 5.4 (qPCR)	(5, 8, 11-14)
<i>Salmonella</i> spp. <sup>c</sup>	0.3	0.7	0.4 ± 1.05 2.8 (qPCR)	(5, 10, 11, 13)
<i>Campylobacter</i> spp.	3.2	NA	NA	(15)
<i>Listeria</i> spp. ( <i>L. monocytogenes</i> )	NA	NA	1.9 ± 0.79 (2.4 ± 0.3)	(13)
<i>Legionella pneumophila</i>	NA	NA	-0.9 ± 2.88 (qPCR)	(9)
<i>Enterococcus</i> spp.	0.8 ± 0.55	1.6 ± 2.19	2.3 ± 1.61, 2.5 ± 0.30 (qPCR)	(4-6, 8, 10-14, 16, 17)
<i>Clostridia</i> spp. ( <i>C. perfringens</i> )	-0.02 ± 0.35 0	-0.4 NA	2.1 ± 0.37 3.5 ± 0.55	(5, 7-9) (6, 11, 13)
( <i>C. difficile</i> )	NA	NA	2.5 (qPCR)	(9)
<b>mean inactivation bacteria<sup>d</sup></b>	<b>1.0 ± 1.1</b>	<b>1.6±1.4</b>	<b>2.6±1.3</b>	
male-specific coliphages	2.1 ± 0.48		3.2 ± 1.61	(7, 9, 14, 16, 18)
somatic coliphages	1.4 ± 1	NA	5.9	(7, 12, 14, 16, 18)
bacteriophages infecting <i>B. fragilis</i>	1.1 ± 0.3	NA	4.5	(7, 14, 16]
enteroviruses	1.3 ± 0.37 1.2 (qPCR)	2.8	2.2 ± 1.07 0.9 (qPCR)	(4, 7, 13, 19-21)
adenovirus	NA	NA	2.4 ± 1.74 (qPCR)	(9)
<b>mean inactivation viruses<sup>d</sup></b>	<b>1.5±0.4</b>	<b>2.8</b>	<b>4.0±1.6</b>	

<sup>a</sup>Log inactivation was based on culturable concentrations (MPN, CFU, PFU) except when indicated by (qPCR), NA= no analyses reported, ND = not detected  
<sup>b</sup>mean and standard deviation of different studies.  
<sup>c</sup>*Salmonella* spp. in TPAD and COM were typically below detection limits. Log reduction values for *Salmonella* spp. in TPAD and COM use the detection limit as a lower bound and therefore underestimate reduction in full-scale plants.  
<sup>d</sup>Only reductions in culturable or infective concentrations considered  
<sup>e</sup>Total inactivation both phases  
<sup>f</sup>COM only inactivation (i.e. no contribution from MAD)

## SUMMARY OF HEALTH EFFECTS STUDIES

**TABLE S2. Summary of Pathogen Epidemiological Studies**

study design	study findings and worst case predicted risk	conclusions	comments
<p><b>Title: “Municipal sewage sludge application on Ohio farms: Health effects”</b> <sup>(22)</sup></p> <p>•Matched prospective epidemiological study investigating the effect of municipal sewage sludge application on the human health of Ohio farmers &amp; family members (n=164). Includes controls comprised of farmers &amp; their families living in areas where no sludge was applied (n=130)</p>	<p>•Tuberculin skin test and sero-conversion against 23 enteroviruses.</p> <p>•Monthly health questionnaires on human and animal health</p>	<p>•Incidences of respiratory and digestive illness as well as general health symptoms were not significantly different between study and control farm residents.</p> <p>•Frequency of sero-conversions was similar in both study populations.</p>	<p>•To date, this study is the most comprehensive epidemiology study on class B biosolids land application.</p> <p>•The authors noted that the absence of elevated risk may have been related to the low application rates used (2-10 dry metric tons/ha, once per year). Authors cautioned against extrapolation of results to other scenarios.</p>
<p><b>Title: “Health survey of residents living near farm fields permitted to receive biosolids”</b> <sup>(23)</sup></p> <p>•Clustered cross sectional survey of mailed health questionnaires including 437 responses from those living on or within 1 mile of a permitted application site and 176 responses from those living more than 1 mile from a biosolids permitted site.</p>	<p>•Considered symptoms in 12 months prior to survey.</p> <p>•Requested information on gastrointestinal, respiratory and general health symptoms as well as chronic diseases.</p>	<p>•Authors observed statistically significant increase in reporting of respiratory, gastrointestinal, and general symptoms in homes less than one mile from permitted class B fields. A statistically relevant negative association between distance and symptoms was found for bronchitis.</p> <p>•In homes near permitted land application sites, significantly greater reporting of acute disease was observed for bronchitis, pneumonia, upper respiratory infections, and giardiasis</p>	<p>•As study limitations, the authors list recall bias and potential errors in self-reporting from residents living near fields due to odors, which may make residents prone to over reporting symptoms.</p>

**TABLE S3. Summary of Biosolids Residential Risk Studies**

study design	study findings and worst case predicted risk	Comments
<p><b>Title:</b> "A risk assessment of emerging pathogens of concern in the land application of biosolids" <sup>(24)</sup></p> <p>•Infectious enteric virus concentrations were produced for a class B wastewater treatment facility, a range of accidental ingestion from 50 mg/day to 480 mg/day was considered. Between 1 and 10 days of exposure per year were considered.</p>	<p>•Maximum risk of infection for rotavirus was <math>2.11 \times 10^{-1}</math> and <math>1.22 \times 10^{-2}</math> for Echovirus-12.</p>	<p>•Authors conclude that incorporation of viruses into soils within 24 hours can significantly reduce risk from ingestions.</p> <p>•Authors also noted that the inability of current methods to detect all of the enteric viruses present in biosolids would lead to an underestimation of infectious risks.</p>
<p><b>Title:</b> "Ecological risk assessment: Bioaerosol transport modeling and risk assessment in relation to biosolids placement" <sup>(25)</sup></p> <p>•Authors used both a Gaussian plume point source model and an area source model described by Parker and coworkers <sup>(26)</sup> to estimate the risk of pathogenic viruses and bacteria aerosolized from large biosolids piles during loading (point source) and from fields during land application (area source). Data used in calculating the source concentrations was taken from a 1997 bioaerosol sampling study at the same site <sup>(27)</sup>. The biosolids application rate was <math>2700 \text{ kg/4} \times 10^3 \text{ m}^2</math> and dewatered anaerobically digested biosolids were applied.</p>	<p>•Annual bacteria (<i>Salmonella</i> spp.) risk of infection for point source aerosols at 100 m (shortest setbacks considered) and 2 m/s wind speed ranged from <math>1.9 \times 10^{-2}</math> for 1 hr exposure to <math>1.2 \times 10^{-1}</math> for an 8 hour exposure.</p> <p>•Annual virus (Coxsackie A21) risk of infection for point source aerosols at 100 m (shortest setbacks considered) and 2 m/s wind speed ranged from <math>3.0 \times 10^{-2}</math> for 1 hr exposure to <math>1.9 \times 10^{-1}</math> for an 8 hour exposure</p>	<p>•Given that many set back distances from land application generally start at 30 m, the risks calculated at 100 m in this year 2000 study are considered very high in accordance with US EPA yearly acceptable risk levels for infection in drinking water of <math>1 \times 10^{-4}</math>. A later 2004 review article from the same research group retracted the risk values for viruses by noting that an incorrect infectivity factor "r" was used in the risk analysis for viruses <sup>(28)</sup>. Using the corrected infectivity factor, as well as a different value of human virus to bacteriophage ratio (0.2 PFU/g human viruses to <math>10^5</math> PFU/g phages in lieu of the 0.2 -200 PFU/g human virus to <math>10^4</math> PFU/g phages used in the year 2000 study), and by using a value of 1 PFU/m<sup>3</sup> phage aerosolized for every 1000 PFU/g (dry) of biosolids to estimate the number of viruses in air, they conclude that the risk of viral infection during biosolids loading (point source) during the worst case scenario (20 m/s wind, 24 hours of exposure) was <math>1.51 \times 10^{-5}</math> for viruses. The previous risk value under the same scenario was dramatically higher <math>9.07 \times 10^{-1}</math>. Both the correction of the infectivity factor, as well as the change of parameters for determining airborne virus concentration resulted in a lower calculated viral risk. The original bacterial risks from the 2000 paper were not revised in this 2004 paper.</p>

**Title: “Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically driven transport model”<sup>(29)</sup>**

•Groundwater was seeded with MS2 coliphage and *E. coli* and applied to land using a biosolids spray application truck. The experiment simulates land application of liquid biosolids. To calculate risk, the empirical decay model was used to first determine phage concentrations at a specific distance from the source. Next, coliphage aerosol concentrations versus distance were transformed into human virus values (enterovirus) by first reducing the coliphage aerosol concentration by 3 to 4 orders of magnitude to account for the lower concentration of coliphages in biosolids than the seeded sample, and then multiplied by  $10^{-6}$  to  $10^{-4}$  to account for the fraction of human viruses/g (dry) (enteroviruses) to coliphage PFU/g (dry) biosolids.

•Under an 8 hour exposure, annual risk of viral infection to residents 30.5 m downwind was  $1.2 \times 10^{-7}$  assuming the  $10^{-6}$  ratio (of animal viruses to coliphages) and  $1.2 \times 10^{-5}$  assuming the  $10^{-4}$  ratio of animal viruses to phages in biosolids.

•For bacteria, The *E. coli* seeded in the sprayer at approximately  $10^5$  CFU/ml was not detectable in aerosols thus the authors concluded that there is little risk from bacteria under this scenario.

•In addition to the inability to measure *E. coli* in downwind aerosols, a major difference in bacterial risk between in this study and the Dowd 2000 study appears to be the ratio of infectious bacteria to indicator bacteria used. The 1:10,000 to 1:1,000,000 range suggested by Brooks and coworkers<sup>(30)</sup> resulted in a aerosol source concentration of less than 1 MPN/m<sup>3</sup> and a low risk, In contrast measurements used by Dowd and coworkers<sup>(25, 27)</sup> in the 2000 study resulted in a maximum airborne *Salmonella* spp. concentration of  $4.1 \times 10^3$  MPN/m<sup>3</sup> and a high risk. We note that this latter *Salmonella* spp. aerosol concentration is atypically high.

• The method used to obtain human virus concentrations from bulk biosolids information was to convert coliphage concentrations to human virus concentrations. Previously, ratios ranging from 1:10,000 to 1:1,000,000 human enterovirus to coliphage have been used by assuming a 1 MPN/dry g concentration of enterovirus viruses<sup>[24]</sup> in class B biosolids and a  $10^5$  PFU/dry gram coliphage concentration. Using the compiled coliphage concentrations from **Table 1** of this manuscript ( $\sim 10^5$  PFU/dry gram of male specific and somatic coliphages) and enterovirus concentration (140 to 1.4 MPN/dry gram for enteroviruses) in **Table 1**, however, yields an updated human virus to total coliphage ratio that is closer to 1:1,000 to 1:100,000 for class B biosolids. By adding adenovirus and norovirus, the true animal virus to coliphage ratio is significantly lower than 1:1,000. Adenovirus and norovirus concentrations were not available at the time of the above study.

**Title: “A national study on the residential impact of biological aerosols from the land application of biosolids”<sup>(30)</sup>**

•A survey of biosolids aerosol risk at ten different land application sites was conducted for *Salmonella* spp. and Coxsackie’s virus A21. Loading operations and spreading operations were considered.

•The max. yearly risk of infection corresponded to loading and was greatest at 30.5 m downwind. Calculated risks were  $3.77 \times 10^{-4}$  and  $1.36 \times 10^{-4}$  for *Salmonella* spp. and Coxsackie’s virus A21 respectively for an 8 hour exposure assumed to occur 6 days in one year<sup>(30)</sup>.

•These results extend the author’s previous risk paper using MS2 and phage to a variety of land application equipment and liquid or cake biosolids at sites around the U.S.

•The same human viral pathogen to phage ratios previously used were also employed in this study.

**Title: "A dynamic model to assess microbial health risks associated with beneficial uses of biosolids" (2)**

•A methodology for assessing risk of illness due to pathogen exposure from biosolids using a population-based model that accounts for secondary transmission and immunity. A case study was carried out for enterovirus exposure via direct consumption of biosolids-amended soils.

•The case study yielded insight into the important factors for quantifying risk. These include biosolids treatment effectiveness, pathogen shedding rate of infected individuals, secondary transmission, and immunity.

•This study extends risk assessment of pathogens beyond chemical-based models to include microbial-specific paths of exposure such as secondary person-to-person exposure. It also considers the importance of population immunity and the importance of pathogen shedding.

•These considerations will improve the accuracy of microbial risk assessments but currently suffer from a lack of data that is needed to enable accurate estimates of factors such as secondary transmission and immunity.

**Title: "Microbial risk assessment framework for exposure to amended sludge projects" (3)**

•Risk analysis completed for direct ingestion, aerosol inhalation, ground water ingestion, and secondary transmission. Pathogen content in biosolids was modeled using pathogen data from raw sludge, treatment efficacy data, and post treatment monitoring data. Risk corresponded to exposure from a single land application event.

•Direct ingestion (rotavirus)  
Depending on class B stabilization treatment: mean risk ranged from  $1 \times 10^{-3}$ ,  $\pm 3 \times 10^{-3}$  st. dev. to  $2 \times 10^{-4} \pm 1 \times 10^{-3}$  std dev.

•Inhalation: a 30 m setback mean aerosol risk was  $2 \times 10^{-4}$

•Groundwater ingestion: Using a 30 m buffer zone from well to biosolids application, the mean risk was  $5 \times 10^{-8}$

•Secondary exposure: Residential plus occupational risk for secondary exposure was mean  $3 \times 10^{-3} \pm 4 \times 10^{-3}$

•Authors conclude that a risk based approach can be used to guide land application practice and to examine the benefits of changing treatment processes and application practices.

•First risk study to include a structure to estimate a range of risks based on uncertainty in parameters.

•Concluded that secondary risk could be as significant as primary risk.

•Analysis limited to enterovirus (reovirus data)

•General ranking of risks was direct ingestion>aerosol inhalation>groundwater ingestion

**Title: "QMRA(quantitative microbial risk assessment) and HACCP (hazard analysis and critical control points) for management of pathogens in wastewater and sewage treatment and reuse" (31)**

•Authors promote the use of a HACCP-based approach for preventative management and quality assurance of

Risk from bacterial and viral agents for an accidental exposure (2 g ingestion, single event corresponding to a child playing at a biosolids storage site)

1. Hemorrhagic *E. coli*,  $1 \times 10^{-2}$
2. *Salmonella* spp.,  $6 \times 10^{-4}$
3. *Giardia* spp.,  $2 \times 10^{-2}$

•Unusually high exposure values used for different scenarios with limited justification for use. Although authors state that some risk numbers are corroborated by examples at plants where workers have chronic infections.

•The study expands risk of viruses beyond enterovirus to include adenovirus.

•Researchers also ranked exposure and risk according to severity of consequences based on the endemic level of these diseases in the community. In this case, adenovirus was listed



biosolids handling and land application rather than random monitoring of indicator and pathogen content in finished biosolids. Risk estimated for rotavirus, adenovirus, <i>Giardia</i> spp., <i>Cryptosporidium</i> spp., <i>Salmonella</i> spp, and hemorrhagic <i>E. coli</i> .	4.	<i>Cryptosporidium</i> spp., $6 \times 10^{-3}$	as a moderate to minor hazardous exposure while exposures to hemorrhagic <i>E. coli</i> and <i>Cryptosporidium</i> spp. were considered major or catastrophic under some exposure scenarios.
	5.	Rotavirus, $4 \times 10^{-1}$	
	6.	Adenovirus, $9 \times 10^{-1}$	

Title: “**Land application of treated sewage sludge: quantifying pathogen risks from consumption of crops**” <sup>(32)</sup>

•Quantitative risk assessments were conducted to estimate the number of humans in the UK infected through consumption of root crops grown on land treated with sewage sludge.

•The probability of infections from *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *E. coli* 0157, *Cryptosporidium parvum*, *Giardia* spp., and enteroviruses was assessed.

•The annual risk level for *Giardia* spp. was  $4.3 \times 10^{-5}$  per year

All others less than  $4.0 \times 10^{-7}$

•Even under worst case scenario (no decay of pathogens in soils), there was a predicted 50 *Giardia* infections per year in England and less than one infection per year from other agents considered.

•Using a required 12 month harvest interval required in the U.K. and assuming a linear decay of pathogens for that 12 months eliminates risk from all agents.

## ESTIMATING AEROSOL PATHOGEN EXPOSURE AND RISK

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. Of the bacterial pathogens with quantitative class B biosolids content information, only *Legionella* spp. has documented incidence of infection via inhalation, however, no inhalation does-response model for *Legionella* spp. in human exists. We conducted QMRA on *Salmonella* spp. based on its importance in setting biosolids regulations <sup>(33)</sup> and its previous consideration in

aerosol risk assessments (**Figure 2**). Viral pathogens that meet all three criteria include adenovirus 4 (representative of adenoviruses), coxsackievirus A21 (representative of enteroviruses), and norovirus. For the exposure scenario modeled here, the total inhalable biosolids dose, pathogen dose, and probability of infection was calculated for the spreading and disking of a square 16 hectare (400 m x 400 m) field with an application rate of 16 dry tons biosolids/ha—this is an average annual sludge application rate for cotton (*Gossypium hirsutum* L.) and is based on agronomic typical N requirements <sup>(34)</sup>. Atmospheric conditions were considered for daytime atmospheric stability classes A through C and wind velocities from 1.5 to 20 m/s. Biosolids spreading was modeled with a ProTwin side-slinger applying at a rate of 0.11 dry metric tons/minute. The spreading ‘puff’ time model applied biosolids to the field four times employing a tractor speed of 0.46 m/s and making 40, 10 m wide (width of side-slinging plume) tractor passes, resulting in a total application time of 38.8 hr. For the same field, the disking tractor moved at 2.2 m/s and made 68, 6-m wide tractor passes (length of disking apparatus) resulting in a total disking time of 3.3 hours.

The inhalable pathogen dose for a land application event was reconstructed for pathogens and pathogen indicators as follows (**Equation 1**):

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

This approach applies the following model components: (i) a Gaussian plume model to estimate downwind biosolids aerosol concentrations under variable emission scenarios and atmospheric stability conditions ( $C$ ,  $\mu\text{g}/\text{m}^3$ ), (ii) an intermittent ‘puff’ exposure time (ET) model to determine exposure time based on spreading and disking equipment movement, wind velocity, and plume dispersion, and (iii) aerosol reconstruction to convert bulk biosolids pathogen concentrations

( $C_{\text{bulk pathogen}}$ ) to an aerosolized pathogen concentration. The breathing rate considered here is for an average adult human (70 kg) at rest with nasal breathing ( $2.3 \times 10^{-4} \text{ m}^3/\text{s}$ ).

Downwind biosolids aerosol concentrations were determined with the Gaussian plume atmospheric transport model (**Equation 2**):

$$C(x, y, z) = \frac{SER}{2\pi u \sigma_y \sigma_z} \exp\left\{-0.5\left(\frac{y}{\sigma_y}\right)^2\right\} \left\{ \exp\left[-0.5\left(\frac{z-H}{\sigma_z}\right)^2\right] + \exp\left[-0.5\left(\frac{z+H}{\sigma_z}\right)^2\right] \right\} \quad [2]$$

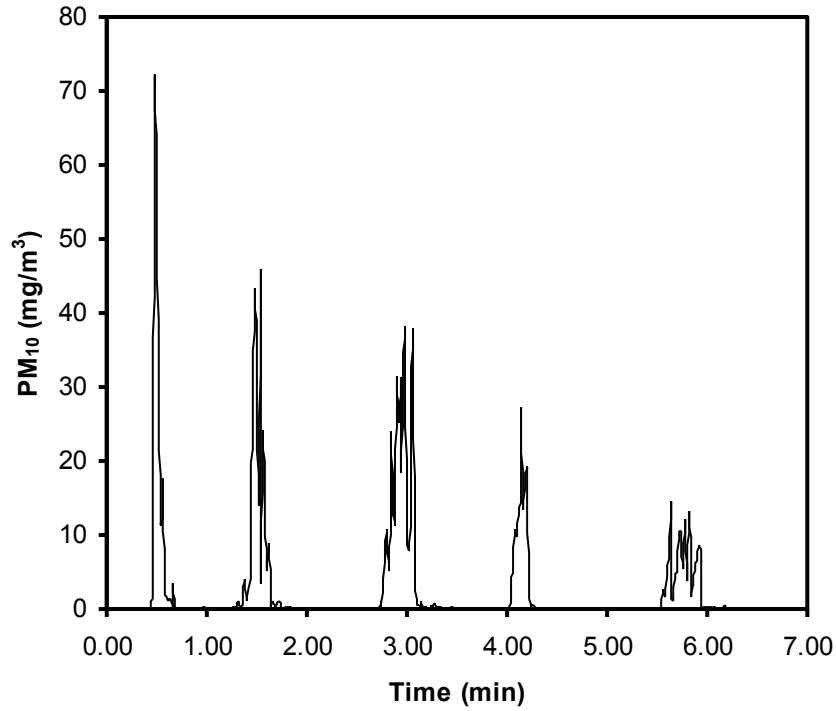
where  $C$  = biosolids  $\text{PM}_{10}$  concentration at  $x$ ,  $y$ , and  $z$  meters downwind from the source,  $x$  = distance downwind (m),  $y$  = horizontal distance perpendicular to wind (m),  $z$  = downwind receptor breathing zone height (1.5 m),  $SER$  = source emission rate ( $\mu\text{g}$  respirable biosolids/s),  $H$  = height of plume source from ground level (m),  $u$  = wind velocity at  $H$  (m/s),  $\sigma_y$  = horizontal dispersion coefficient (m) and  $\sigma_z$  = vertical dispersion coefficient (m). The major input to the Gaussian model is a source emission rate ( $SER$ ).  $SER$ 's have previously been independently estimated for spreading and for diking of dewatered biosolids by multiplying a measured aerosol source concentration gradient ( $\mu\text{g}$  biosolids  $\text{PM}_{10}/\text{volume}$ ) by a volumetric airflow (volume air/time) through the source plume cross-sectional area perpendicular to the wind direction.  $SER$ 's for spreading of dewatered biosolids by side-slinging<sup>(35)</sup> and disk incorporation of dewatered biosolids into soil<sup>(36)</sup> were  $10,100 \pm 8000 \mu\text{g}$  biosolids  $\text{PM}_{10}/\text{s}$  and  $18,600 \mu\text{g}$  biosolids  $\text{PM}_{10}/\text{s}$ , respectively. To account for aerosol inactivation, a log decay with respect to time was assumed. Due to the uncertainty in virus aerosol inactivation rates, ranges were used. For log decay rates of enteroviruses, the upper limit of aerosol inactivation was based on poliovirus inactivation and set at  $2.29 \text{ log/hr}$  (45% RH)<sup>(37)</sup>. At 165 m downwind and a low 1.5 m/s wind speed, only a 15% reduction in infective concentration would be expected. Thus, as a conservative estimate, the lower level inactivation for enteroviruses was set at zero. The upper

limit of adenovirus aerosol inactivation was based on bovine adenovirus inactivation and set at 2.4 log/hr (30% RH) <sup>(38)</sup>. Similar to enteroviruses, the lower level of aerosol inactivation for adenovirus was set at zero. Finally, the upper limit of Norovirus inactivation was based on a feline calicivirus study and was set at 6 log/hr (40% RH) <sup>(39)</sup>. The lower level of aerosol inactivation for norovirus was set at zero.

The next input to the **Equation 1** inhalation dose formula was exposure time. For disking and spreading, exposure does not occur continuously, but will be an intermittent series of aerosol “puffs” that occur each time the application and disking equipment move past a stationary downwind receptor (**Figure S1**). An intermittent ‘puff’ exposure time model was previously developed from PM<sub>10</sub> measurements during disking operations <sup>(40)</sup> and accounts for wind speed, spreading or disking equipment speed, and the effect of atmospheric dispersion on the puff’s dimensions. Total exposure time is the summation of the series of individual puff exposure times (**Equation 3**):

$$ET = \sum \frac{L}{u} = \sum \frac{2 \times k \sigma_y}{u} \quad [3]$$

where ET = exposure time for a downwind receptor, L = length of the aerosol puff in the x-direction (m), and u = wind velocity (m/s). The length of one side of the puff is calculated with the horizontal dispersion coefficient  $\sigma_y$  (m) and the coefficient k (unitless) that indicates the percentage of the plume mass considered in each puff. Based on best fits to puffs measured in the field using real-time PM<sub>10</sub> monitors, we use k = 3 to contain 99.73% of the plume mass <sup>(40)</sup>. As the plume disperses with distance, single puff exposure times at long distances and low wind speeds may exceed the time between puffs and thus become continuous. Once this occurs, the exposure time term is change from a puff-based time to a continuous time.

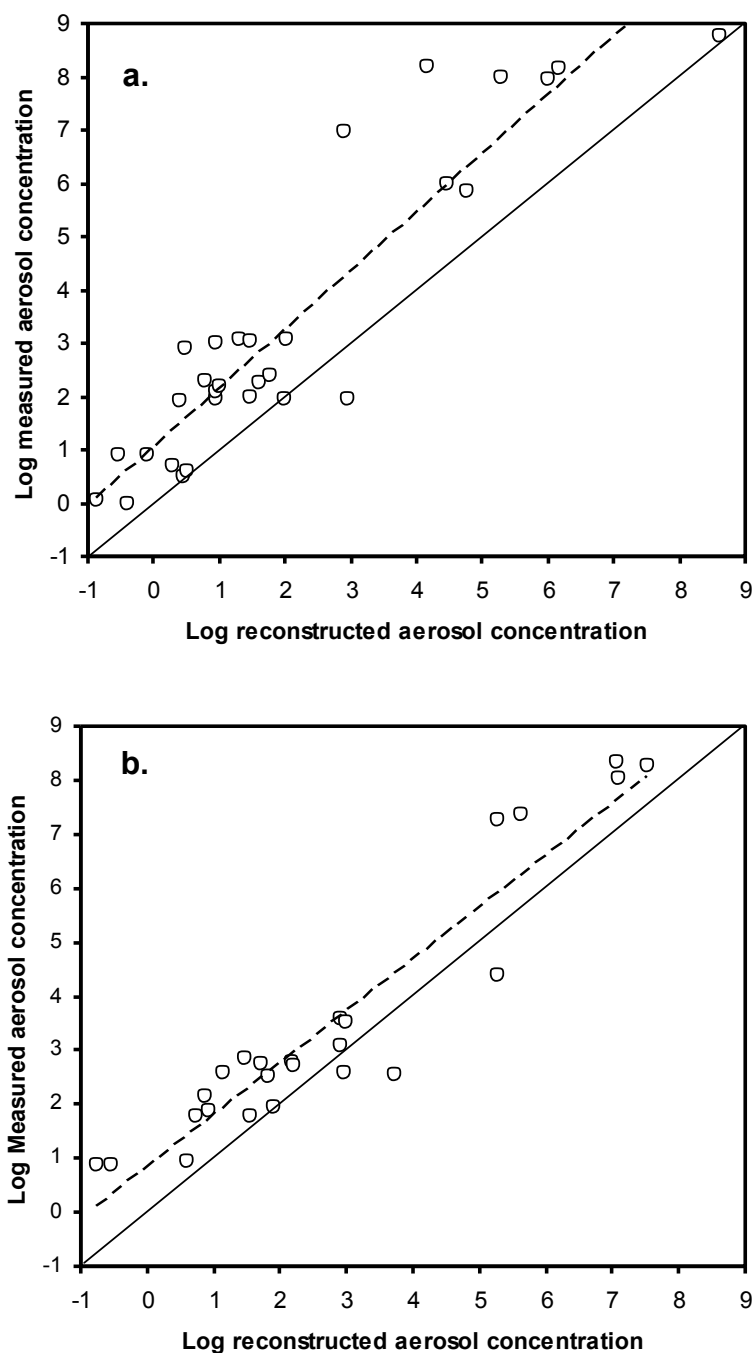


**Figure S1.** Real-time measures of  $PM_{10}$  emitted during the disking of land applied biosolids demonstrates the characteristic intermittent nature of the plume to a stationary receptor. The first peak corresponds to disking at the source ( $\sim 5$  m from a  $PM_{10}$  Monitor monitor (DustTrak™, Shoreview, MN) placed at a 1.5 m height) and successive peaks indicate sequential passes of the disking equipment.

Next, aerosol doses of respirable biosolids were converted to aerosolized pathogen dose using an empirically validated aerosol reconstruction technique (**Equation 4**).

$$D_{\text{aerosolized pathogen}} (\text{pathogen unit}) = D_{\text{respirable biosolids}} (\mu\text{g}) \times C_{\text{pathogen in bulk biosolids}} \left( \frac{\text{pathogen unit}}{\mu\text{g}} \right) \quad [4]$$

where,  $D_{\text{aerosolized pathogen}}$  is the inhalation dose of pathogens (#),  $D_{\text{respirable biosolids}}$  is the inhalation dose of biosolids ( $\mu\text{g}$ ), and  $C_{\text{pathogen in bulk biosolids}}$  is the concentration of pathogens in the bulk sludge ( $\#/\mu\text{g}$ ). **Figure S2** demonstrates the basis for using reconstruction for aerosols produced



**Figure S2.** Aerosol reconstructed concentrations versus aerosol measured concentrations for (a) biosolids diking and (b) biosolids spreading. Values represent concentrations of metals, endotoxins, and different classes of indicator and total microorganisms. Before log transformation, aerosol concentrations are number per  $\text{m}^3$  for biological concentrations, and mass per  $\text{m}^3$  for chemical aerosol concentration. Aerosols were measured as total suspended particles. Dashed lines represent the best linear fit with the corresponding  $r^2$  value and slope. Plots modified from <sup>(41)</sup>.

during spreading and disking. Values in **Figure S2** were measured at the aerosol source where biosolids indicators can be more easily measured. Here, analytically derived biological and chemical aerosol values were compared to values produced from reconstruction of biological and chemical agents over a broad concentration range. To add pathogen data for quantitative microbial risk assessment (QMRA), the concentrations within all studies were compiled and the range (maximum and minimum) of concentrations was used. Reporting the overall range allows for employing a log-uniform distribution of pathogen content in subsequent risk estimations. The use of this distribution for highly variable parameter ranges and pathogen contents is consistent with previous QMRA's <sup>(42, 43)</sup>. For class B biosolids, the minimum infectious enterovirus concentration was set at -1.37 log PFU/gram and the maximum concentration was set at 2.06 log PFU/ gram <sup>(24, 44)</sup>. Enterovirus occurrence in class B biosolids has previously been estimated to be 100% <sup>(45)</sup>. The minimum total adenovirus concentration for class B biosolids was set at 2.28 log GU/dry gram <sup>(46)</sup> and the maximum concentration was set at 7.9 log GU/dry gram <sup>(9)</sup>. It has previously been noted that approximately 88% of class B biosolids samples test positive for adenovirus<sup>[9]</sup>. A uniform range of infective to total adenovirus of 1/1,000 to 1/10,000 viruses was used to estimate the infectious adenovirus particles <sup>(47)</sup>. The minimum norovirus virus concentration was set at 2.5 log GU/dry gram and the maximum virus concentration was set at 7 log GU/g dry gram <sup>(45)</sup>. Norovirus GI and GII values were utilized. Occurrence of above detection limit concentrations of norovirus in class B biosolids has been estimated at 67% <sup>(45)</sup>. The efficiencies of extracting viruses from sludges are included in the reported concentrations for norovirus and adenovirus <sup>(9, 45)</sup>, but not typically in enterovirus. High and low values for enteroviruses were therefore adjusted by estimating a uniform range of extraction efficiencies from 10% to 100%. The minimum *Salmonella* spp. concentration was set at 1 MPN/dry gram

and the maximum 3.66 log GU/dry gram <sup>(48, 49)</sup>. Bacterial aerosol inactivation was modeled as a uniform range from 0 to 13.68 log/hr <sup>(50)</sup>. *Salmonella* spp. occurrence has previously been measured in 27% of class B biosolids samples <sup>(27)</sup>. Pathogen ranges and aerosol inactivation ranges were modeled as a log-uniform distribution and values produced using a Monte Carlo simulation, 10,000 trials.

Finally, and to estimate risk, the aerosol pathogen concentration exposures are converted to probability of infection by applying the pathogen-specific dose-response model and parameters. Yearly probability of infection values are presented for adult individuals. Although there may be differences within healthy adults, dose-response parameters are not available for immunocompromised populations or children. For enteroviruses, respiratory infection was estimated based on an existing single-hit exponential dose-response model for coxsackievirus A21. An  $r$  value = 0.0253 <sup>(29, 51)</sup> was used and it was assumed that every viral particle was infectious <sup>(29)</sup>. For adenovirus, respiratory infection was estimated as the health endpoint and a fraction of the qPCR-based concentration was assumed to be infectious as described above. The single-hit exponential model was utilized with an  $r$  = 0.4172 for adenovirus, originally derived from aerosol exposure to adenovirus 4 <sup>(52)</sup>. For norovirus, a gastrointestinal infection was estimated as the health endpoint and a 2F1 confluent Gaussian hypergeometric distribution was used <sup>(53)</sup>. This model has three set input parameters, alpha and beta dose response parameters, and an aggregation parameter  $a$ . An alpha value of 0.04 and a beta value of 0.055 were used. A source of uncertainty for norovirus is whether it is appropriate to assume the particles are aggregated or disaggregated in environmental matrices. To include this uncertainty in the analysis, results for both disaggregated ( $a=0.0001$ ) and aggregated ( $a=0.999$ ) are presented. The probability of infection for norovirus was calculated using the “hypergeom” function in Matlab 7



(Mathworks, Natick, MA USA). For norovirus, which causes gastrointestinal infections and where the airborne route of infection has been observed, the dose-response model for ingestion was used along 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. <sup>(30)</sup>. Here we use a more conservative range in values of 10% to 50% and Monte Carlo simulations using 10,000 trials to define the range. Finally, for *Salmonella* spp., gastrointestinal infection was estimated as the health endpoint. A Beta-Poisson dose-response model was utilized with an alpha value of 0.3126 and an N50 value of 23,600 MPN <sup>(54)</sup>. A range of 10% to 50% of inhaled bacterial particles were assumed to be ingested <sup>(30)</sup>. **Tables 2, S4, and S5** present the biosolids dose, the pathogen dose, and the probability of infection, respectively for different atmospheric stabilities, wind speeds, and set back distances.

TABLE S4. Enterovirus, Adenovirus, and Norovirus Log Inhalation Dose (Log # infectious particles)					
Distance to Downwind Receptor (m)	Dose (log #) at 1.5 m/s, atmos. stab. class A	Dose (log #) at 3 m/s, atmos. stab. class B	Dose (log #) at 6 m/s, atmos. stab. class C	Dose (log #) at 10 m/s, atmos. stab. class C	Dose (log #) at 20 m/s, atmos. stab. class C
<b>Enterovirus</b>					
5	-4.13	-4.51	-4.95	-5.38	-6.00
30	-4.19	-4.59	-5.02	-5.45	-6.07
65	-4.29	-4.69	-5.13	-5.57	-6.17
165	-4.58	-4.88	-5.30	-5.76	-6.35
500	-5.25	-5.25	-5.60	-6.05	-6.63
1000	-5.80	-5.60	-5.82	-6.27	-6.83
<b>Adenovirus</b>					
5	-2.90	-3.28	-3.72	-4.15	-4.77
30	-2.96	-3.37	-3.79	-4.22	-4.84
65	-3.06	-3.46	-3.90	-4.34	-4.94
165	-3.36	-3.65	-4.07	-4.53	-5.12
500	-4.02	-4.02	-4.38	-4.82	-5.40
1000	-4.57	-4.38	-4.59	-5.04	-5.60
<b>Norovirus</b>					
5	0.27	-0.41	-1.19	-1.96	-3.06
30	0.16	-0.57	-1.32	-2.08	-3.18
65	-0.02	-0.73	-1.51	-2.29	-3.36
165	-0.55	-1.07	-1.81	-2.63	-3.68
500	-1.72	-1.72	-2.37	-3.15	-4.18
1000	-2.70	-2.37	-2.74	-3.54	-4.53

For enterovirus, a concentration of 2.96 PFU/dry gram (the median of study values) and an extraction efficiency of 100% were assumed. For adenovirus, a concentration of  $10^{4.7}$  GU/dry gram and 1/1000 infectious particles was assumed. For Norovirus, a concentration of  $10^5$  GU/dry gram was assumed.

**TABLE S5. Log Yearly Median Probability of Infection.**

<b>Distance to Downwind Receptor (m)</b>	<b>Risk at 1.5 m/s, atmos. stab. class A</b>	<b>Risk at 3 m/s, atmos. stab. class B</b>	<b>Risk at 6 m/s, atmos. stab. class C</b>	<b>Risk at 10 m/s, atmos. stab. class C</b>	<b>Risk at 20 m/s, atmos. stab. class C</b>
<b>Enterovirus</b>					
5	-5.72	-6.10	-6.55	-6.98	-7.59
30	-5.79	-6.19	-6.62	-7.05	-7.66
65	-5.89	-6.29	-6.73	-7.17	-7.76
165	-6.18	-6.47	-6.90	-7.35	-7.95
500	-6.85	-6.85	-7.20	-7.65	-8.22
1000	-7.39	-7.20	-7.42	-7.87	-8.43
<b>Adenovirus</b>					
5	-4.60	-4.98	-5.42	-5.85	-6.47
30	-4.66	-5.07	-5.49	-5.92	-6.54
65	-4.76	-5.16	-5.60	-6.04	-6.64
165	-5.06	-5.35	-5.77	-6.23	-6.82
500	-5.72	-5.72	-6.08	-6.52	-7.10
1000	-6.27	-6.08	-6.29	-6.74	-7.30
<b>Norovirus (disaggregated)</b>					
5	-0.43	-0.86	-1.58	-2.33	-3.43
30	-0.48	-1.00	-1.70	-2.46	-3.56
65	-0.58	-1.14	-1.89	-2.67	-3.74
165	-0.98	-1.46	-2.19	-3.01	-4.06
500	-2.10	-2.10	-2.74	-3.52	-4.55
1000	-3.08	-2.74	-3.11	-3.91	-4.91
<b>Norovirus (aggregated)</b>					
5	-2.12	-2.80	-3.58	-4.34	-5.45
30	-2.23	-2.96	-3.70	-4.47	-5.57
65	-2.41	-3.12	-3.90	-4.68	-5.75
165	-2.94	-3.46	-4.20	-5.02	-6.07
500	-4.11	-4.11	-4.75	-5.54	-6.57
1000	-5.09	-4.75	-5.13	-5.93	-6.92

## References

1. 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.
2. Eisenberg, J.; Soller, J.; Scott, J.; Eisenberg, D.; Colford, J., A dynamic model to assess microbial health risks associated with beneficial uses of biosolids. *Risk Analysis* **2004**, *24*, 221-236.
3. 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 Persp.* **2008**, *116*, 727-733.
4. 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*, (2), 361-368.
5. Briancesco, 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.
6. 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.
7. Guzman, C.; Jofre, J.; Montemayor, M.; Lucena, F., Occurrence and levels of indicators and selected pathogens in different sludges and biosolids. *Journal of Applied Microbiology* **2007**, *103*, 2420-2429.
8. 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.
9. 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.
10. Watanabe, T.; Kitamura, T.; Ochi, S.; Ozaki, M., Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Water Sci. Technol.* **1997**, *36*, 25-32.
11. 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.
12. 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*, 3763-3770.
13. 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.
14. 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.
15. Jones, K.; Betaieb, M.; Telford, D. R., Seasonal variation of thermophilic campylobacters in sewage sludge. *J. Appl. Bacteriol.* **1990**, *69*, 185-189.

16. 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.
17. Viau, E.; Peccia, J., Evaluation of the enterococci indicator in biosolids using culture-based and quantitative PCR assays. *Water Res.* **2009**, *43*, 4878-4887.
18. Mignotte-Cadiergues, B.; Gantzer, C.; Schwartzbrod, J., Evaluation of bacteriophages during the treatment of sludge. *Water Sci. Technol.* **2002**, *46*, 189-194.
19. 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.
20. Schwartzbrod, L.; Mathieu, C., Virus recovery from wastewater treatment plant sludges. *Water Res.* **1986**, *20*, 1011-1013.
21. 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.
22. 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.
23. 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. H.* **2007**, *62*, 5-11.
24. Gerba, C.; Pepper, I.; Whitehead, L., A risk assessment of emerging pathogens of concern in the land application of biosolids. *Water Sci. Technol.* **2002**, *46*, 225-230.
25. 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.
26. Parker, D. T.; Spendlove, J. C.; Bondurant, J. H., Microbial aerosols from food-processing waste spray fields. *J. Water Poll. Contr. Fed.* **1977**, *49*, 2359-2365.
27. 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.
28. Brooks, J. P.; Gerba, C. P.; Pepper, I., Biological Aerosol Emission, Fate, and Transport from Municipal and Animal Wastes. *J. Residuals Sci. Tech.* **2004**, *1*.
29. 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.
30. 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.
31. 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.
32. Gale, P., Land application of treated sewage sludge: quantifying pathogen risks from consumption of crops. *J. Appl. Microbiol.* **2005**, *98*, 380-396.
33. Yanko, W. A., Occurrence of pathogens in distribution and marketing municipal sludges. Report # EPA/600/S1-87/014. 1988, EPA Office of Research and Development, Research Triangle North Carolina.

34. Zerzghi, 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.
35. 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*, (10), 3537-3544.
36. 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. *Atm. Environ.* **2006**, *40*, 7034-7045.
37. Jong, J. C. d.; Winkler, K. C., The Inactivation of Poliovirus in Aerosols. *J. Hygiene* **1968**, *66*, 557-565.
38. Elazhary, M. A.; Derbyshire, J. B., Aerosol stability of bovine adenovirus type 3. *Can. J. Comp. Med.* **1979**, *43*, (3), 305-12.
39. Donaldson, A. I.; Ferris, N. P., The survival of some air-borne animal viruses in relation to relative humidity. *Vet. Microbiol.* **1976**, *1*, 413-420.
40. 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.
41. Peccia, J.; Paez-Rubio, T., *Quantification of airborne biological contaminants associated with land applied biosolids. Report 02-PUM-1*. IWA London, 2007.
42. Eisenberg, J. N. S.; Seto, E. Y. W.; Colford, J. M.; Olivieri, A. W.; Spear, R. C., An analysis of the Milwaukee cryptosporidiosis outbreak based on a dynamic model for the infection process. *Epidemiology* **1998**, *9*, 255-263.
43. 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.
44. Goddard, M. R.; Bates, J.; Butler, M., Recovery of indigenous enteroviruses from raw and digested sewage sludges. *Applied and Environ. Microbiol.* **1981**, *42*, 1023-1028.
45. 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.
46. 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.
47. He, J.-W.; Jiang, S., Quantification of Enterococci and Human Adenoviruses in Environmental Samples by Real-Time PCR. *Appl. Environ. Microbiol.* **2005**, *71*, 2250-2255.
48. 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.
49. Pillai, S.; Widmer, K.; Dowd, S.; Ricke, S., Occurrence of Airborne Bacteria and Pathogen Indicators during Land Application of Sewage Sludge. *Appl. Environ. Microbiol.* **1996**, *62*, 296-299.
50. Teltsch, B.; Shuval, H.; Tadmor, J., Die-Away Kinetics of Aerosolized Bacteria from Sprinkler Application of Wastewater. *Appl. Environ. Microbiol.* **1980**, *39*, 1191-1197.

51. Couch, R. B.; Douglas, R. G.; Lindgren, K. M.; Gerone, P. J.; Knight, V., Airborne Transmission of Respiratory Infection With Coxsackievirus A Type 21. *Am. J. Epidemiol.* **1970**, *91*, 78-86.
52. Knight, V., Airborne transmission and pulmonary deposition of respiratory viruses. *In Fourth International Symposium on Aerobiology*, Hers, J. F. P.; Windler, K. C., Eds. Oosthoek, Utrecht, The Netherlands, 1973; pp 175-182.
53. 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.
54. Haas, C.; JB, R.; Gerba, C., *Quantitative microbial risk assessment*. John Wiley & Sons, Inc: New York, 1999; p 449.