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# A national study on the residential impact of biological aerosols from the land application of biosolids

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## ABSTRACT

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**Aims:** The purpose of this study was to evaluate the community risk of infection from bioaerosols to residents living near biosolids land application sites.

**Methods and Results:** Approximately 350 aerosol samples from 10 sites located throughout the USA were collected via the use of six SKC Biosamplers®. Downwind aerosol samples from biosolids loading, unloading, land application and background operations were collected from all sites. All samples were analysed for the presence of HPC bacteria, total coliform bacteria, *Escherichia coli*, *Clostridium perfringens*, coliphage, enteroviruses, hepatitis A virus and norovirus. Total coliforms, *E. coli*, *C. perfringens* and coliphage were not detected with great frequency from any sites, however, biosolids loading operations resulted in the largest concentrations of these aerosolized microbial indicators. Microbial risk analyses were conducted on loading and land application operations and their subsequent residential exposures determined.

**Conclusions:** The greatest annual risks of infection occurred during loading operations, and resulted in a $4 \times 10^{-4}$ chance of infection from inhalation of coxsackievirus A21. Land application of biosolids resulted in risks that were $<2 \times 10^{-4}$ from inhalation of coxsackievirus A21. Overall bioaerosol exposure from biosolids operations poses little community risk based on this study.

**Significance and Impact of the Study:** This study evaluated the overall incidence of aerosolized micro-organisms from the land application of biosolids and subsequently determined that microbial risks of infection were low for residents close to biosolids application sites.

**Keywords:** aerosol, bioaerosol, biosolids, pathogens, risk.

## INTRODUCTION

Concerns about the link between biological aerosols associated with the land application of biosolids and the incidence of illness within neighbouring residences has received recent public attention in the USA (Fackelmann 2002). In the USA, over 50% of the 6 million metric tons (dry) of the biosolids produced are land applied as class B biosolids (National Research Council: Committee on Toxicants and Pathogens in Biosolids Applied to Land 2002). Class B biosolids have been treated either physically (anaerobic digestion) or chemically (lime addition) to reduce faecal coliforms to a concentration below 2 million most probable number (MPN) $g^{-1}$. The Environmental Protection Agency (EPA) established regulations regarding the treatment, disposal, and reuse of biosolids as a fertilizer to protect human health, however, bioaerosol generation was not well addressed (Environmental Protection Agency 1994; National Research Council: Committee on Toxicants and Pathogens in Biosolids Applied to Land 2002).

A limited number of studies have been conducted on the generation of bioaerosols from biosolids land application. Notably, Sorber *et al.* (1984) concluded that little or no risk

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was associated with the land application of liquid biosolids based on the lack of pathogenic viral presence in large volumes of sampled air. Other studies have focused on large piles of biosolids, unloaded by trucks on site, and subsequently loaded with front-end loaders into biosolids spreaders or hoppers (Pillai *et al.* 1996; Dowd *et al.* 2000). Loading events proved to be sources of increased concentrations of faecal microbial indicators such as, $H_2S$ producing bacteria, and *Clostridium* spp. No risk analyses were conducted in the former study although the investigators concluded that the microbial indicator concentrations were below levels that could be construed as a risk to public health. The latter study conducted microbial risk analyses based on the use of complex transport models first proposed for the transport of chemical aerosols (Pasquill 1961). Through the use of these models, aerosol concentrations could effectively be predicted at downwind distances from both point (biosolids pile) and area sources (a biosolids applied field) (Dowd *et al.* 2000). Conservative occupational risk analysis was conducted and risk calculations ranged from a 3% chance of infection to a 100% chance of infection based on infection from aerosolized coxsackievirus.

This present study was conducted to evaluate the microbial concentrations within biological aerosols at several class B biosolids land application sites throughout the USA. Both cultural and molecular techniques were applied to determine microbial concentrations of indicator bacteria, coliphage and pathogenic enteric viruses. In addition, microbial risk analyses were conducted to determine the risk of infection.

## MATERIALS AND METHODS

### Sample sites and biosolids application

A total of 10 sites across the continental USA were sampled including: Marana, AZ; Eloy, AZ; Picacho, AZ; Mojave, AZ; Solano, AZ; Snoqualmie, WA; Sunnyside, WA; Leesburg, VA; Houston, TX and Chicago, IL. Sites were chosen to encompass varied environmental conditions such as: low/high relative humidity, low/high temperature and variable windspeed. Samples were collected from February 2002 to August 2003 (Table 1).

Methods of biosolids application, type and treatment as per site are listed in Table 1. Application procedures also influenced site selection as multiple methods of application are available and practiced throughout the country. Specifically, most types of class B biosolids involved in this study were at least 15% dry mass 'cake' biosolids (thickened biosolids), although sites such as Houston, TX applied liquid 2% dry mass class B biosolids. Thickened biosolids application involved either, 'spreading' or 'slinging' techniques. Throughout this study 'spreading' of biosolids consisted of using a modified manure spreader or 'slinging' which involved the action of launching the biosolids hundreds of feet into the air. Liquid biosolids were applied through the use of spray tankers or irrigation techniques.

### Sample strategy for aerosol collection

Due to the differences in biosolids application found at each site, different strategies were employed for sample collection. 'Cake' biosolids application lent itself to multiple sample collection opportunities including: 'loading', 'slinging', 'spreading' and truck 'unloading' operations. Liquid biosolids application allowed for sample collection only during truck spray applications and irrigation processes.

Samples collected during loading events are described here as processes that involved the loading of class B 'cake' biosolids into an application device via the use of a front-end loader. Samplers were placed downwind and perpendicular to the wind vector and direction of loading. Samples collected during application events are designated as slinging or spreading events. This entailed the physical land application of the biosolids. As this approach involved a moving point source, samplers were aligned parallel to the travel vector and perpendicular to the wind speed vector. Samples collected during unloading stages involved the unloading of the biosolids on-site typically from a 'dump truck' directly onto the soil or vegetation.

Aerosol samples were collected from either downwind placements or upwind placements (background) samples. Background samples were collected during conditions of minimal soil disturbance while no biosolids operations were being conducted. Overall samplers were placed three per specific downwind distance at two separate distances per operation, comprising one round of sample collection. Samples collected during loading or unloading events at 2 m and greater distances were directly downwind of these events, whereas during application operations, a 2-m sample or greater distance refers to downwind of the biosolids application perimeter.

*Sites 1, 2, and 3: Marana, Eloy and Picacho, AZ.* Liquid biosolids were applied to cotton fields from a BetterBuilt® spray tanker (Better Built Equipment, Alpharetta, GA, USA) at each of these sites. Aerosol samples were collected during this event.

*Site 4: Mojave, AZ.* 'Cake' biosolids were land applied to cotton fields via the use of a Knight Protwin® Slinger (Kuhn Knight Inc., Brodhead, WI, USA). Biosolids were launched from the applicator *c.* 30 m into the air. This approach provided two different opportunities for sample collection, specifically samples were collected from loading and slinging operations.

**Table 1** Sample sites visited throughout the study and associated biosolids application method

| Site | Location | Collection dates | RH (%) | Temperature (°C) | WS m s$^{-1}$ | Type of biosolids | Application method |
|---|---|---|---|---|---|---|---|
| 1 | Marana, AZ | 8 Feb 2002 to 19 Feb 2003 | 20·0 | 16·0 | 2·1 | Class B Anaerobic Liquid (7–8%) | Spray tanker Betterbuilt |
| 2 | Eloy, AZ | 21 Mar 2002 to 6 Jun 2002 | 15·6 | 21·8 | 1·5 | Class B Anaerobic Liquid (7–8%) | Spray tanker Betterbuilt |
| 3 | Picacho, AZ | 19 Jun 2002 | 11·5 | 25·2 | 1·5 | Class B Anaerobic Liquid (7–8%) | Spray tanker Betterbuilt |
| 4 | Mojave, AZ | 16 Jul 2002 to 19 Jul 2002 | 37·5 | 34·3 | 1·1 | Class B Anaerobic Cake (21%) | Slinger Knight Protwin Slinger |
| 5 | Solano, CA | 6 Aug 2002 to 8 Aug 2002 | 40·4 | 22·1 | 2·5 | Class B Anaerobic Cake (20%) | Manure Spreader |
| 6 | Snoqualmie, WA | 13 Jan 2003 to 15 Jan 2003 | 75·6 | 8·0 | 0·3 | Class B Anaerobic Cake (16%) | Slinger Aerosopread |
| 7 | Sunnyside, WA | 25 Mar 2003 to 27 Mar 2003 | 41·4 | 13·8 | 2·1 | Class B Anaerobic Cake (27·6%) | Slinger Knight Protwin Slinger |
| 8 | Leesburg, VA | 6 May 2003 to 7 May 2003 | 54·3 | 18·5 | 0·7 | Class B Anaerobic Cake (24%) | Slinger Knight Protwin Slinger |
| 9 | Houston, TX | 6 Aug 2003 to 7 Aug 2003 | 39·8 | 36·5 | 2·3 | Class B Anaerobic Liquid (2%) | Spray Irrigation |
| 10 | Chicago, IL | 20 Aug 2003 | 54·4 | 19·8 | 1·6 | Class B Anaerobic Cake (17%) | Spreader AgChem TerraGator |

RH, relative humidity; WS, Windspeed.

*Site 5: Solano, CA.* 'Cake' biosolids were land applied to grass pasture lands via the use of a modified manure spreader. Through the action of the manure spreader, biosolids were applied from *c.* 1 m above the ground and 10 m behind the apparatus. Aerosol samples were collected from loading, spreading and truck unloading operations.

*Site 6: Snoqualmie, WA.* Aerosol samples were collected from a biosolids application site, in which 'cake' biosolids were applied to local tree farms. Biosolids were launched into the tree tops via the use of a Fecon Aerospreader® (Fecon Inc., Cincinnati, OH, USA), modified for the application of biosolids. Samples were collected during loading, and slinging operations. Specifically at this site during loading operations, biosolids were first unloaded into a metal bin used to store the biosolids, and subsequently loaded into the biosolids applicators using a modified log forwarder scoop.

*Site 7: Yakima, WA.* Hop fields were applied with 'cake' biosolids via the use of a Knight Protwin® biosolids slinger (Kuhn Knight Inc.). Samples were collected from both 'loading' and 'slinging' operations.

*Site 8: Leesburg, VA.* Samples were collected from a grass pasture field, to which 'cake' biosolids were land applied. Biosolids were applied via the use of a Knight Protwin® slinger (Kuhn Knight Inc.) with samples collected during loading operations.

*Site 9: Houston, TX.* Samples were collected from a grass pasture field, to which 2% liquid biosolids were land applied through the use of an irrigation sprinkler. Biosolids were spread in a circular fashion as the irrigator operated in a rotating motion, with a radius of *c.* 10 m. Samples were collected during the spray application events.

*Site 10: Chicago, IL.* Cake biosolids were land applied to a grass pasture field via the use of a modified AgChem Terragator® manure spreader (AgCo, Jackson, MN, USA). Samples were collected following application events, in which biosolids were land applied 2–3 days prior to aerosol sample collection.

### Aerosol and biosolids sample collection

Biological aerosol samples were collected via the use of six SKC Biosamplers® (SKC-West Inc., Fullerton, CA, USA). Vac-U-Go® sampling pumps (SKC-West Inc.) were employed to provide a constant air sampling rate of 12·5 l min$^{-1}$. All samples were collected at a height of 1·5 m, set atop of aluminum tripods (Seco Mfg., Redding, CA, USA) (ASTM 2004a). Samples were collected for a total of 20 min or *c.* 250 l of sampled air. Biosamplers were loaded with 23 ml of 0·1% peptone buffer amended with antifoam agent B (Sigma-Aldrich, St Louis, MO, USA). Following sample collection, all were placed on ice and transported overnight for analysis. Prior to analysis, samples were brought back to volume (23 ml) with 0·1% peptone buffer and vortexed for 1 min. Weather conditions were monitored through the use of a Kestrel portable weather monitor (Nielsen-Kellerman, Boothwyn, PA, USA).

In addition to aerosol samples, composite biosolids samples were collected from each site, placed on ice and transported for analysis. From this composite sample, 10-g (moist) biosolids were dried in a convection oven at 104°C for 24 h to ascertain solid percentage and hence dry mass. All data was reported on a dry weight basis. Prior to analysis, moist biosolids samples were homogenized by placing 10 g into 95 ml 0·1% peptone water. This peptone water extract mixture was shaken in a Labline multiwrist shaker (Barnstead Int., Dubuque, IA, USA) for 30 min on medium setting, and serially diluted to accommodate HPC, *Clostridium perfringens*, total coliform and *Escherichia coli* assays. Liquid biosolids samples were serially diluted from the above mentioned sample mixture for coliphage detection. In contrast, 'cake' biosolids samples were extracted via the use of beef extract following the recommended ASTM (2004b) procedure for the extraction of human enteric viruses from thickened biosolids. The eluted solution was then used to carry out coliphage assays.

### Microbial assays

*HPC.* Aerosolized heterotrophic plate count (HPC) bacteria were assayed in triplicate utilizing R2A media via the spread plate method. An aliquot of the aerosol sample (0·1 ml), including serial dilutions were spread onto R2A media (Becton Dickinson, Sparks, MD, USA) and incubated at 25°C for 7 d. R2A facilitated the enumeration of potentially damaged aerosolized bacteria. Biosolids samples were assayed in the same manner. An aliquot of the peptone water extract was serially diluted and assayed as described above. Aerosol samples were reported as colony-forming units (CFU) m$^{-3}$, and biosolids samples were reported as CFU g$^{-1}$.

*Coliphage.* Aerosolized coliphage able to infect *E. coli* ATCC 15597 was assayed utilizing the double agar overlay technique (Adams 1959). A total of 4 ml from the aerosol sample was assayed using this method. To assay biosolids samples, a 1-ml aliquot of serially diluted sample extract was screened via the double agar overlay technique. In addition to this, incubation times were reduced from 24 h for aerosol samples to 16 h for biosolids samples to avoid overgrowth of background bacteria. Aerosol samples were reported as plaque-forming units (PFU) m$^{-3}$, and biosolids samples were reported as PFU g$^{-1}$.

*Total coliform and* E. coli. Aerosolized total coliform bacteria and *E. coli* were assayed utilizing the commercially available Colilert® enzyme assay (IDEXX; Westbrook, ME) coupled with the Quantitray® Most Probable Number method (American Public Health Association, American Water Works Association, and Water Environment Federation 1998). A total of 5 ml of the aerosol sample was assayed utilizing this method. Total coliforms and *E. coli* were quantified from biosolids through the use of the serially diluted peptone water extract. As in the aerosol samples this liquid extract was assayed via the use of Colilert® enzyme assay coupled with Quantitray® (IDEXX, Westbrook, ME, USA). Aerosol samples were reported as most probable number (MPN) m$^{-3}$, and biosolids samples were reported as MPN g$^{-1}$.

Clostridium perfringens. *Clostridium perfringens* was assayed using membrane filtration onto modified mCP media (Acumedia Manufacturers, Baltimore, MD, USA) (Arnon and Payment 1988). All samples were heat shocked at 70°C for 20 min prior to sample analysis. Heat shocking results in enumeration of clostridia spores as vegetative cells are inactivated through the use of heat (Arnon and Payment 1988). A total of 5 ml of the aerosol sample was filtered through a membrane filter (0·45 $\mu$m) and aseptically transferred to the media. Petri dishes were then incubated for 1–2 d at 44·5°C in an anaerobically sealed jar (Becton Dickinson Microbiology Systems, Sparks, MD, USA), with anaerobic conditions provided by GasPak Plus (Becton Dickinson Microbiology Systems). Biosolids samples were assayed for the presence of *C. perfringens* via the use of serially diluted peptone water extract, in the same fashion as the aerosol samples. Aerosol samples were reported as CFU m$^{-3}$, and biosolids reported as CFU g$^{-1}$.

*Molecular techniques: enterovirus, HAV, norovirus.* Reverse transcriptase polymerase chain reaction (RT-PCR) was the chosen method of analysis to determine human pathogenic virus presence or absence within the bioaerosols. Following sample collection, an 8-ml portion of the aerosol sample was frozen at −20°C. Prior to extraction of RNA, this aliquot was first concentrated using commercially available Centriprep 50 concentrators (Millipore, Billerica, MA, USA) operating at a speed of 1500 *g* for 5 min followed by a second spin of 1000 *g* for 5 min. This yielded a final volume between 0·6 and 1 ml. In addition to these samples, select aerosol samples were concentrated in their entirety (23 ml) to a final concentrate between 0·6 and 1 ml. RNA was extracted using commercially available Qiagen viral RNA extraction kits (Qiagen, Valencia, CA, USA) as described by the manufacturer. An aliquot of 280 $\mu$l of concentrated sample was extracted using these kits and concentrated to a final volume of 80 $\mu$l.

This final concentrate potentially containing viral RNA was then assayed for the presence of enteroviruses, noroviruses and hepatitis A virus nucleic acid. Amplification was carried out on an Applied Biosystems Geneamp PCR system 2700 (Applied Biosystems, Foster City, CA, USA).

*Enteroviruses/hepatitis a virus RTPCR protocol.* RTPCR was performed through the use of Qiagen One Step RTPCR kits (Qiagen) under the following conditions: RNA was transcribed via a single pre-PCR step of 30 min at 50°C, followed by a single step of 15 min at 95°C. A three-step PCR process, 35 cycles total, began with a cDNA denature step performed at 94°C for 45 s; primer annealing was performed at 53°C for 30 s, followed by DNA extension at 72°C for 1 min. All reagents were provided through the Qiagen One Step RTPCR kit, and were added in concentrations recommend by manufacturer's specifications. Primers were provided by Sigma Genosys (The Woodlands, TX, USA), with previously described sequences (Schwab *et al.* 1996) to amplify a 197- and 192-bp product for enteroviruses and hepatitis A virus respectively. A final primer concentration of 0·6 $\mu$mol $l^{-1}$ was achieved. A final volume of 40 $\mu$l with 10 $\mu$l of template constituted the final tube volume of 50 $\mu$l.

Following initial amplification, a second amplification was performed to increase sensitivity (Alvarez *et al.* 1995). An internal product of 105 bp was produced from enterovirus PCR templates using an internal primer provided by Schwab *et al.* (1996) coupled with the upstream primer. Hepatitis A virus amplicons were amplified via the use of a second reamplification, employing both primers from the original PCR. A 10-$\mu$l aliquot of the previously amplified product was added to fresh master mix containing and amplified under the following conditions: a single pre-PCR initial AmpliTaq-Gold® (Applied Biosystems, Foster City, CA, USA) activation step of 10 min at 95°C, followed by 30 cycles of amplification, denature step of 30 s at 95°C and a combined primer annealing/extension step of 72°C for 45 s followed by a final extension step of 72°C for 10 min. Reagents were added in the following concentrations and volumes for enterovirus secondary amplification: sterile PCR water (28·45 $\mu$l), 10X PCR buffer II (Applied Biosystems) (5 $\mu$l), 25 mmol $l^{-1}$ $MgCl_2$ (Applied Biosystems) (5 $\mu$l), 10 mmol $l^{-1}$ DNTP solution (1 $\mu$l), 5 U $\mu l^{-1}$ Amplitaq Gold® (Applied Biosystems) (0·25 $\mu$l) and each primer 200 $\mu$mol $l^{-1}$ (0·15 $\mu$l) to constitute a final volume of 50 $\mu$l. Reagents were added in the following concentrations and volumes for HAV secondary amplification: sterile PCR water (32·0 $\mu$l), 10X PCR buffer II (4·5 $\mu$l), 25 mmol $l^{-1}$ $MgCl_2$ (2·4 $\mu$l), 10 mmol $l^{-1}$ DNTP solution (0·5 $\mu$l), 5 U $\mu l^{-1}$ Amplitaq Gold (0·30 $\mu$l) and each primer 200 $\mu$mol $l^{-1}$ (0·15 $\mu$l) to constitute a final volume of 50 $\mu$l. All samples were analysed in duplicate.

*Norovirus RTPCR protocol.* Qiagen One Step RTPCR kits were used with modifications as described by Vinje *et al.* (2004). Volumes described were doubled to accommodate larger sample volumes. A reamplification step was included to increase sensitivity, which consisted of 10 $\mu$l being removed from the original amplification and added to fresh master mix and amplified under the following conditions: a single pre-PCR initial Taq-Gold activation step of 10 min at 95°C, followed by 30 cycles of amplification, denature step of 30 s at 95°C, primer annealing of 30 s at 50°C and an extension step of 72°C for 30 s followed by a final extension step of 72°C for 10 min. Reagents were added in the following concentrations and volumes: sterile PCR water (30·5 $\mu$l), 10X PCR buffer II (4·5 $\mu$l), 25 mmol $l^{-1}$ $MgCl_2$ (2·4 $\mu$l), 10 mmol $l^{-1}$ DNTP solution (0·5 $\mu$l), 5 U $\mu l^{-1}$ Amplitaq Gold (0·5 $\mu$l), and 50 $\mu$mol $l^{-1}$ primer MJV12 (1·0 $\mu$l), 50 $\mu$mol $l^{-1}$ primer RegA (0·6 $\mu$l) to constitute a final volume of 50 $\mu$l. All samples were analysed in duplicate.

*Visualization.* An aliquot of 10 $\mu$l of the final double round PCR product was loaded into a 1·6% agarose gel and visualized via ethidium bromide staining and UV illumination. Any PCR-positive samples were sequenced via an on campus DNA sequencing facility following purification with a QIAquick PCR purification system (Qiagen). Sequences were analysed via the Blast program available on the internet (http://www.ncbi.nlm.nih.gov).

## Statistical analysis

Statistical analyses, specifically analysis of variance were performed via the use of Minitab statistical analysis program (Mintab Inc., State College, PA, USA).

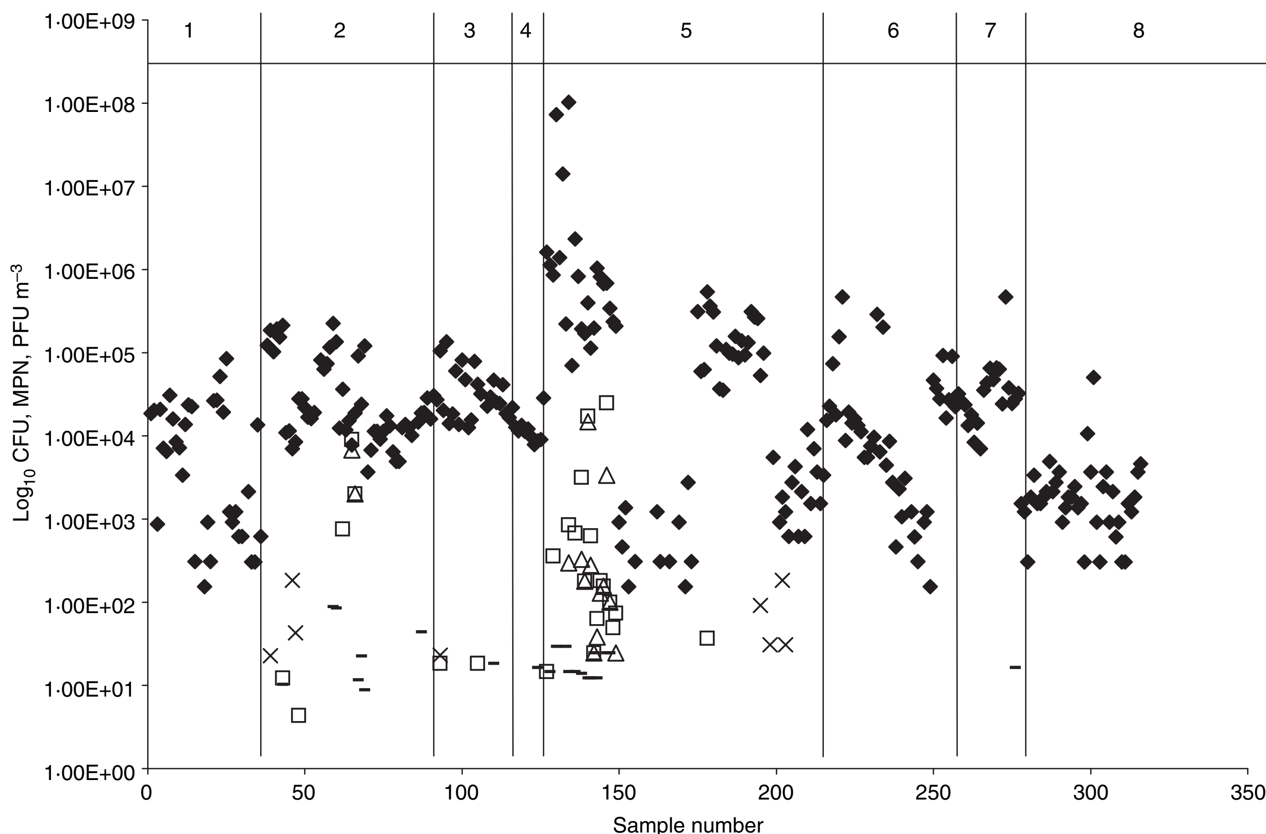


**Fig. 1** Aerosol microbial concentrations for each microbe assayed from sites 1–10, listed per biosolids operation. HPC, heterotrophic plate count bacteria. Detection limits = HPC 307 CFU $m^{-3}$, TotCol 18 MPN $m^{-3}$, *Escherichia coli* 18 MPN $m^{-3}$, *Clostridium perfringens* 18 CFU $m^{-3}$, and coliphage 23 PFU $m^{-3}$. Aerosol samples collected: 1, background; 2, spray truck; 3, spray irrigation; 4, unloading; 5, loading; 6, slinging; 7, spreading; 8, postapplication samples; (◆) aerosolized HPC; (□) aerosolized total coliforms; (△) aerosolized *E. coli*; (×) aerosolized coliphage; (—) aerosolized *C. perfringens*

## RESULTS

### Samples

Figures 1 and 2 show aerosol and biosolids sample microbial concentrations.

### Biosolids samples

Collection of class B biosolids from multiple sites throughout the country showed that samples were similar in microbial quantity and quality. With the exception of two samples (Houston, TX/Leesburg, VA, USA), most biosolids samples contained the following approximate concentrations: HPC bacteria ($10^9$ $g^{-1}$), total coliforms ($10^5$–$10^6$ $g^{-1}$), *E. coli* ($10^4$ $g^{-1}$), presumptive *C. perfringens* ($10^5$–$10^6$ $g^{-1}$) and coliphage ($10^4$ $g^{-1}$).

### Aerosol samples

*Biosolids spray tanker application.* Aerosol samples collected from sites 1, 2 and 3 in southern Arizona, all

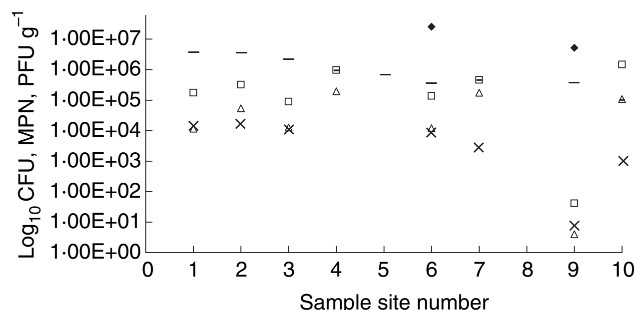


**Fig. 2** Class B biosolids microbial concentrations per sampling site per dry g. (◆) HPC; □ total coliforms; (△) *Escherichia coli*; (×) coliphage; (—) *Clostridium perfringens*

demonstrated concentrations of indicator microbes at or below detectable levels. Samples were collected between 2 and 20 m downwind of liquid biosolids application. Overall, HPC bacteria were detected at levels greater than background concentrations, *c.* 0·5 $\log_{10}$ greater, which was statistically significant ($P < 0{\cdot}05$). At distances of 20 m, HPC aerosol concentrations were statistically similar to

background samples. Total coliform bacteria, *C. perfringens* and *E. coli* were detected occasionally, but were not detected frequently, and only at distances within 5 m downwind of the operation. No aerosolized coliphage was detected. No pathogenic enteric viruses were detected via RTPCR.

*Biosolids spray irrigation.* Site 9 consisted of aerosol samples collected downwind of 2% liquid biosolids spray irrigation. All samples contained concentrations of HPC bacteria, *c.* 0·5 $\log_{10}$ greater than most background samples collected. *Clostridium perfringens*, total coliforms and coliphage were detected at distances of 11 and 40 m. This was the greatest distance from the application site that coliphage had been detected throughout this study, however, the detection of these indicator microbes was inconsistent, as only a few samples were positive. Pathogenic viruses were not detected through the use of RTPCR.

*'Cake' biosolids operations.* The majority of aerosol samples collected in this study were collected downwind of 'cake' biosolids land application, as this process is the most commonly used throughout the USA. Through this operation, samples were collected from loading, slinging, spreading, unloading, background and following application operations.

*'Cake' biosolids spreading.* Sites 5 and 10 involved aerosol collection from operations in which 'cake' biosolids were spread via modified manure spreaders. Site 5 samples were collected downwind of loading, unloading and spreading sites, whereas site 10 samples were collected 2 d postapplication of biosolids. Site 5 HPC concentrations from loading processes were statistically elevated over background, unloading and spreading concentrations ($P < 0{\cdot}05$). Total coliforms, *E. coli* and *C. perfringens* were all detected during loading processes. Total coliform bacteria were detected in all samples collected from loading sites at distances between 2 and 15 m, although concentrations decreased by 2 $\log_{10}$ to $10^2$ MPN $m^{-3}$ at 15 m ($P < 0{\cdot}05$). Similar results were obtained from *E. coli* aerosol concentrations downwind of loading situations. *Clostridium perfringens* was detected at low concentrations from loading events and was often barely above detection limits. 'Unloading' events yielded *C. perfringens* upon one occasion, but no other indicator micro-organisms were detected. Aerosolized HPC bacteria were detected at concentrations similar to background concentrations as no statistical difference was noted between unloading and background aerosol samples. Spreading operations yielded *C. perfringens* on one occasion only, while HPC bacteria were detected at *c.* 0·5 $\log_{10}$ greater than background HPC concentrations. These concentrations decreased to levels similar to background concentrations ($P < 0{\cdot}05$) beyond 28 m. No pathogenic viruses, or coliphage were detected from this site.

Site 10 consisted of samples collected from post application sites, in which biosolids were land applied 2 d prior to aerosol sample collection. Throughout this sampling period, no indicator bacteria or coliphage were detected in any aerosol samples, and overall HPC concentrations were at levels similar to typical background concentrations.

*'Cake' biosolids slinging.* Sites 4, 6, 7 and 8 involved sample collection from biosolids land application sites involving slinger operation. Loading samples collected between distances of 2 and 10 m from site 4 contained elevated levels of indicator bacteria such as total coliforms, *E. coli* and *C. perfringens* although none were elevated at a statistically significant level. HPC bacteria concentrations were greater than background concentrations, and often times were 2 $\log_{10}$ greater than background levels. HPC aerosol concentrations involved with loading scenarios were significantly greater than those from slinging samples, which were found to only contain HPC bacteria at concentrations 0·5 $\log_{10}$ greater than background concentrations. It is important to note that of all the sites visited throughout this study, site 4 was the only site to have produced PCR-positive samples, two of which were detected during 'slinging' samples and one collected during 'loading' samples at 5 and 2 m respectively. The three positive samples contained norovirus nucleic acid as sequenced from PCR-positive samples. No coliphage was detected at this site.

Site 6 samples were collected from slinger land application operations. It is important to note that samples were collected from a moist wooded area in the Pacific northwest, which ultimately affected overall levels of aerosolized micro-organisms often times reducing HPC bacterial concentrations below detectable levels. During both loading and slinging situations only HPC bacteria were detected. Background concentrations demonstrated no significant difference when compared with loading and slinging situations.

Samples from site 7 were collected from a biosolids slinger operation and consisted of loading and slinging aerosol samples. HPC concentrations during slinging operations were similar to background levels, while 'loading' conditions yielded statistically significant ($P < 0{\cdot}05$) levels, *c.* 0·5 $\log_{10}$ greater than background concentrations. While coliphage and *C. perfringens* were detected during 'loading' operations, neither was detected with frequency nor were any at levels statistically greater than background samples.

Site 8 consisted of samples collected from only loading operations. No significant differences were noted between HPC bacteria from loading and background samples. No indicator bacteria were detected from 'loading' operations although coliphage was detected between distances of 2 and 30 m, but not at significantly greater concentrations than detection limits or with great frequency.

## Microbial risk assessment

To conduct bacterial and viral risk analyses, transport modelling was performed utilizing a previously described transport model (Brooks *et al.* 2005). Although this model describes transport of coliphage from land applied biosolids, it was utilized here to estimate both viral and bacterial transport. This approach is inherently conservative as aerosolized bacteria, specifically Gram-negative bacteria exhibit greater inactivation than coliphage and hence travel less distance (Teltsch *et al.* 1980). This model was used to describe coliform and coliphage transport from 'cake' biosolids application sites, during loading and spreading operations ranging from a baseline distance of 2 to 1000 m.

To model transport of coliform bacteria from biosolids loading, total coliform aerosol concentrations from a loading operation (site 5, 2 m samples or 3·84 $\log_{10}$ MPN $m^{-3}$) were modelled with inactivation rates [0·036 ($\log_{10}$ PFU $m^{-3}$) $m^{-1}$ travelled] from the previously described coliphage transport model. To model coliform bacteria from spreading operations, detection limits (1·26 $\log_{10}$ MPN $m^{-3}$) were utilized as base values at 2 m downwind of a spreading operation. No coliforms were detected during spreading operations, therefore detection limits were used in lieu of actual incidence data. To model coliphage transport from loading and spreading operations, detected coliphage (site 8, 2 m samples or 1·61 $\log_{10}$ PFU $m^{-3}$) and detection limits (1·37 $\log_{10}$ PFU $m^{-3}$) were utilized, respectively, as stated above.

Once transport of indicator bacteria and coliphage was modelled, ratios were applied to estimate aerosolized concentrations of enteric pathogenic bacteria and viruses as previously described (Brooks *et al.* 2005). Ratios ranging from conservative 1 : 10 000 to the least conservative 1 : 1 000 000 (pathogenic bacteria/virus to indicator bacteria/virus) were used to predict aerosolized *Salmonella* spp. and coxsackievirus A21 concentrations at specific downwind distances. These ratios represent a range of expected concentrations for both organisms present in class B biosolids (Gerba *et al.* 2002). In addition these ratios generated pathogen concentrations (enteric viruses, *Salmonella* spp.), which bracket consistently recovered pathogen densities (*c.* 1 MPN 4 $g^{-1}$ enteric viruses, *c.* 50 MPN $g^{-1}$ *Salmonella*) in our laboratory from anaerobically digested class B biosolids (unpublished data). It was assumed that both pathogenic micro-organisms were aerosolized with the same efficiency as the modelled predictions of their aerosolized indicator counterparts.

Risks of infection were determined using the one-hit exponential model for inhalation of coxsackievirus A21 (Haas *et al.* 1999), $P_i = 1 - \exp(-rN)$, and the $\beta$-Poisson infectivity model for ingestion of nontyphoid *Salmonella* spp. (Haas *et al.* 1999):

$$P_i = 1 - \left[\left(1 + \frac{N}{N_{50}}\right)\left(2^{1/\alpha} - 1\right)\right]^{-\alpha}$$

where $r = 0{\cdot}0253$ coxsackievirus A21 (inhalation) (Couch *et al.* 1965), $\alpha = 0{\cdot}3126$ *Salmonella* spp. (nontyphoid) (ingestion) (Haas *et al.* 1999), $N_{50} = 23\ 600$ *Salmonella* spp. (nontyphoid) (ingestion) (Haas *et al.* 1999), and $N$ is the exposure dose in number of organisms.

These models were chosen, as they most accurately describe the likelihood of infection from a one-time coxsackievirus A21 and *Salmonella* spp. aerosol exposure.

The exposure dose is described as

$$N = x \times 0{\cdot}83 \times t,$$

where $x$ is the number of organisms per $m^3$, 0·83 $m^3$ $h^{-1}$ = the average human breathing rate (Environmental Protection Agency 1997), and $t$ is the exposure duration in h.

For coxsackievirus A21 it was assumed that all viruses were inhaled, as an inhalation dose response model exists for this pathogen. However, for *Salmonella* spp. exposures it was assumed that 50% of inhaled micro-organisms were also subsequently ingested, hence dose response based on ingestion was utilized. Some studies have utilized 10% ingestion to calculate exposure to aerosolized faecal bacteria in microbial risk assessment (Medema *et al.* 2004). The bioaerosol diameter at which 50% of all collected bioaerosols ($d_{50}$) using the SKC Biosampler is 0·3 $\mu$m, which when inhaled is typically deposited within the alveolar passages (Stetzenbach 2001). As such, for dose calculations all other bioaerosols (50%) containing bacteria were assumed to be larger than this. These were assumed to be inhaled, trapped in mucus, and subsequently swallowed, hence 50% ingestion.

The annual risk model is described as:

$$P_{(\text{annual})} = 1 - (1 - P_i)^{d},$$

where $P_i$ is the one-time (daily) probability of infection, described above, d is the number of days exposed per year.

As the approach in this study was to focus on residential impact, only residential risks (i.e. community risks) were calculated. Residential exposure here was described as any distance beyond 30·5 m downwind of an application site, as this represents the minimum setback distance between a land application site and a residence (National Research Council: Committee on Toxicants and Pathogens in Biosolids Applied to Land 2002). It is important to note that public exposure from sites 1–10, were located at least 250 m from the land application sites. All risk values have been calculated and presented based on multiple pathogen to indicator ratios, ranging from conservative (1 : 10 000) to least conservative (1 : 1 000 000). Both one-time and annual risks of infection for residences near a biosolids application site were calculated. One-time risks of infection (daily) were calculated based on either a 1- or an 8-h one-time exposure,

**Table 2** Calculated annual risks of infection for aerosolized *Salmonella* spp. and coxsackievirus A21 for biosolids spreading and loading scenarios based on various pathogen to indicator ratios, exposure times in h $d^{-1}$, and downwind (DW) distances

| | Exposure time | | | | | |
|---|---|---|---|---|---|---|
| | 1 : 10 000 | | 1 : 100 000 | | 1 : 1 000 000 | |
| DW distance (m) | 1 h | 8 h | 1 h | 8 h | 1 h | 8 h |
| Annual (6 d $year^{-1}$) Risks of infection *Salmonella* spp. – spreading operations | | | | | | |
| 30·5 | $4{\cdot}54 \times 10^{-8}$ | $3{\cdot}63 \times 10^{-7}$ | $4{\cdot}54 \times 10^{-9}$ | $3{\cdot}63 \times 10^{-8}$ | $4{\cdot}54 \times 10^{-10}$ | $3{\cdot}63 \times 10^{-9}$ |
| 50 | $8{\cdot}84 \times 10^{-9}$ | $7{\cdot}07 \times 10^{-8}$ | $8{\cdot}84 \times 10^{-10}$ | $7{\cdot}07 \times 10^{-9}$ | $8{\cdot}84 \times 10^{-11}$ | $7{\cdot}07 \times 10^{-10}$ |
| 83·9 | $5{\cdot}14 \times 10^{-10}$ | $4{\cdot}11 \times 10^{-9}$ | $5{\cdot}14 \times 10^{-11}$ | $4{\cdot}11 \times 10^{-10}$ | $5{\cdot}14 \times 10^{-12}$ | $4{\cdot}11 \times 10^{-11}$ |
| 100 | $1{\cdot}33 \times 10^{-10}$ | $1{\cdot}06 \times 10^{-9}$ | $1{\cdot}33 \times 10^{-11}$ | $1{\cdot}06 \times 10^{-10}$ | $1{\cdot}33 \times 10^{-12}$ | $1{\cdot}06 \times 10^{-11}$ |
| 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 |
| Annual (6 d $year^{-1}$) risks of infection coxsackievirus A21 – spreading operations | | | | | | |
| 30·5 | $2{\cdot}65 \times 10^{-5}$ | $2{\cdot}12 \times 10^{-4}$ | $2{\cdot}65 \times 10^{-6}$ | $2{\cdot}12 \times 10^{-5}$ | $2{\cdot}65 \times 10^{-7}$ | $2{\cdot}12 \times 10^{-6}$ |
| 50 | $5{\cdot}16 \times 10^{-6}$ | $4{\cdot}13 \times 10^{-5}$ | $5{\cdot}16 \times 10^{-7}$ | $4{\cdot}13 \times 10^{-6}$ | $5{\cdot}16 \times 10^{-8}$ | $4{\cdot}13 \times 10^{-7}$ |
| 83·9 | $3{\cdot}00 \times 10^{-7}$ | $2{\cdot}40 \times 10^{-6}$ | $3{\cdot}00 \times 10^{-8}$ | $2{\cdot}40 \times 10^{-7}$ | $3{\cdot}00 \times 10^{-9}$ | $2{\cdot}40 \times 10^{-8}$ |
| 100 | $7{\cdot}76 \times 10^{-8}$ | $6{\cdot}21 \times 10^{-7}$ | $7{\cdot}76 \times 10^{-9}$ | $6{\cdot}21 \times 10^{-8}$ | $7{\cdot}76 \times 10^{-10}$ | $6{\cdot}21 \times 10^{-9}$ |
| 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 |
| Annual (6 d $year^{-1}$) risks of infection *Salmonella* spp. – loading operations | | | | | | |
| 30·5 | $1{\cdot}70 \times 10^{-5}$ | $1{\cdot}36 \times 10^{-4}$ | $1{\cdot}70 \times 10^{-6}$ | $1{\cdot}36 \times 10^{-5}$ | $1{\cdot}70 \times 10^{-7}$ | $1{\cdot}36 \times 10^{-6}$ |
| 50 | $3{\cdot}31 \times 10^{-6}$ | $2{\cdot}65 \times 10^{-5}$ | $3{\cdot}31 \times 10^{-7}$ | $2{\cdot}65 \times 10^{-6}$ | $3{\cdot}31 \times 10^{-8}$ | $2{\cdot}65 \times 10^{-7}$ |
| 83·9 | $1{\cdot}92 \times 10^{-7}$ | $1{\cdot}54 \times 10^{-6}$ | $1{\cdot}92 \times 10^{-8}$ | $1{\cdot}54 \times 10^{-7}$ | $1{\cdot}92 \times 10^{-9}$ | $1{\cdot}54 \times 10^{-8}$ |
| 100 | $4{\cdot}98 \times 10^{-8}$ | $3{\cdot}98 \times 10^{-7}$ | $4{\cdot}98 \times 10^{-9}$ | $3{\cdot}98 \times 10^{-8}$ | $4{\cdot}98 \times 10^{-10}$ | $3{\cdot}98 \times 10^{-9}$ |
| 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 |
| Annual (6 d $year^{-1}$) risks of infection coxsackievirus A21 – loading operations | | | | | | |
| 30·5 | $4{\cdot}71 \times 10^{-5}$ | $3{\cdot}77 \times 10^{-4}$ | $4{\cdot}71 \times 10^{-6}$ | $3{\cdot}77 \times 10^{-5}$ | $4{\cdot}71 \times 10^{-7}$ | $3{\cdot}77 \times 10^{-6}$ |
| 50 | $9{\cdot}17 \times 10^{-6}$ | $7{\cdot}34 \times 10^{-5}$ | $9{\cdot}17 \times 10^{-7}$ | $7{\cdot}34 \times 10^{-6}$ | $9{\cdot}17 \times 10^{-8}$ | $7{\cdot}34 \times 10^{-7}$ |
| 83·9 | $5{\cdot}33 \times 10^{-7}$ | $4{\cdot}26 \times 10^{-6}$ | $5{\cdot}33 \times 10^{-8}$ | $4{\cdot}26 \times 10^{-7}$ | $5{\cdot}33 \times 10^{-9}$ | $4{\cdot}26 \times 10^{-8}$ |
| 100 | $1{\cdot}38 \times 10^{-7}$ | $1{\cdot}10 \times 10^{-6}$ | $1{\cdot}38 \times 10^{-8}$ | $1{\cdot}10 \times 10^{-7}$ | $1{\cdot}38 \times 10^{-9}$ | $1{\cdot}10 \times 10^{-8}$ |
| 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 |

Annual risks of infection based on 6 days exposure per year. Zero values are below smallest risk calculation represented and are not actual zero risk of infection.

while annual risks of infection were based on these same daily exposures, 6 d $year^{-1}$. A 6-d $year^{-1}$ exposure is assumed to be from two, 3-d biosolids application exposures per year as noted by field observations. Field applications typically take place once per year for *c.* 3 d depending on field size, however to be conservative, two applications were assumed to have taken place, hence 6 d $year^{-1}$. Table 2 presents annual risk of infection from exposure to aerosolized coxsackievirus A21 and *Salmonella* spp. from distances equal to and beyond 30·5 m downwind of a biosolids land application operation.

*Biosolids spreading operation* – Salmonella *spp.* During biosolids spreading operations, a 1 h exposure to aerosolized *Salmonella* spp. resulted in $7{\cdot}57 \times 10^{-9}$ (7·57 chance of infection in one billion or 0·000000757%) risk of infection per exposure, while an 8 h exposure resulted in $6{\cdot}06 \times 10^{-8}$ risk of infection per exposure using the most conservative ratio. Using the least conservative ratio resulted in $7{\cdot}57 \times 10^{-11}$ one-time risk of infection when exposure occurred for 1 h and $6{\cdot}06 \times 10^{-10}$ risk of infection per exposure when exposure occurred for 8 h. Annual risks of infection for residential exposure based on these daily exposures to biosolids spreading operations for 6 d $year^{-1}$ resulted in risks of $4{\cdot}54 \times 10^{-8}$ (1 h $d^{-1}$) annual risk of infection and $3{\cdot}63 \times 10^{-7}$ (8 h $d^{-1}$) annual risk of infection, while less conservative calculations were $4{\cdot}54 \times 10^{-10}$ (1 h $d^{-1}$) and $3{\cdot}63 \times 10^{-9}$ (8 h $d^{-1}$) annual risks of infection.

*Biosolids spreading operations – coxsackievirus A21.* Exposures to coxsackievirus A21 from biosolids spreading operations for 1 h resulted in a range of one-time infectious risk from $4{\cdot}42 \times 10^{-6}$ to $4{\cdot}42 \times 10^{-8}$ from most to least

conservative, while an 8 h exposure resulted in a range of infectious risk from $3{\cdot}53 \times 10^{-5}$ to $3{\cdot}53 \times 10^{-7}$ from most to least conservative. Annual risks of infection from these daily exposures 6 d year$^{-1}$ resulted in $2{\cdot}65 \times 10^{-5}$ annual risk of infection to the least conservative $2{\cdot}65 \times 10^{-7}$ annual risk of infection for a daily 1 h exposure, while daily 8 h exposures resulted in annual risk of infection ranges from $2{\cdot}12 \times 10^{-4}$ to $2{\cdot}12 \times 10^{-6}$.

*Biosolids loading operations –* Salmonella *spp.* One-time risk of infection because of exposure to aerosolized *Salmonella* spp. from biosolids loading resulted in a risk of infection ranging from $2{\cdot}83 \times 10^{-6}$ to $2{\cdot}83 \times 10^{-8}$ per exposure when exposure occurred for 1 h at least 30·5 m downwind from the operation. An 8 h exposure at 30·5 m downwind from the operation resulted in a range from $2{\cdot}27 \times 10^{-5}$ to $2{\cdot}27 \times 10^{-7}$ risk of infection per exposure. Annual risks of *Salmonella* infection based on these 1 and 8 h exposures per day over 6 d year$^{-1}$ resulted in $1{\cdot}70 \times 10^{-5}$ and $1{\cdot}36 \times 10^{-4}$ annual risks of infection, respectively, while the less conservative approach yielded $1{\cdot}70 \times 10^{-7}$ and $1{\cdot}36 \times 10^{-6}$ annual risks of infection respectively.

*Biosolids loading operations – coxsackievirus A21.* Exposure to aerosolized coxsackievirus A21 during loading conditions for 1 and 8 h exposure times resulted in one-time risks of infection of $7{\cdot}85 \times 10^{-6}$ and $6{\cdot}28 \times 10^{-5}$ respectively. Using the less conservative ratio resulted in one-time risks of infection equal to $7{\cdot}85 \times 10^{-8}$ (1 h d$^{-1}$) and $6{\cdot}28 \times 10^{-7}$ (8 h d$^{-1}$) per exposure. Annual risks of infection based on these same daily risks of infection and 6 d year$^{-1}$ exposure resulted in $4{\cdot}71 \times 10^{-5}$ (1 h d$^{-1}$) and $3{\cdot}77 \times 10^{-4}$ (8 h d$^{-1}$), respectively, while the less conservative risk calculation resulted in $4{\cdot}71 \times 10^{-7}$ (1 h d$^{-1}$) and $3{\cdot}77 \times 10^{-6}$ (8 h d$^{-1}$) respectively.

## DISCUSSION

Indicator micro-organisms such as total coliforms, *E. coli*, *C. perfringens*, and coliphage were rarely detected as aerosols, with concentrations typically only slightly above detection limits. The increased detection of indicator bacteria at sites associated with loading operations was noted. Only during biosolids loading operations did total coliforms and *E. coli* regularly reach levels above detection limits, however these micro-organisms were only detected at distances within 15 m of these biosolids sites. In addition some sites had inadvertently incorporated soil into the biosolids during this event, specifically at sites 4 and 5. In this case, soil particles may have protected aerosolized microbes from environmental inactivation factors such as dessication, ultraviolet light, and oxygen radicals (Lighthart and Stetzenbach 1994).

*Clostridium perfringens* was more readily detected during land application and loading events, but detection was limited to distances within 15 m. HPC bacteria were detected readily, with the exception of sites located in areas of high relative humidity where soils were moist, such as sites 6 and 8. Overall, during biosolids operations aerosolized HPC bacteria were one order of magnitude greater than background concentrations ($10^3$ HPC m$^{-3}$), and were regularly found at greater concentrations (>2 $\log_{10}$) than any specific biosolids borne microbe. In addition, HPC bacteria were not readily detected when soil was not incorporated into the biosolids loading (Sites 6 and 8). Soil was not collected along with the biosolids and hence mixed in during front end loader operation. This limited aerosolized HPC concentrations to background concentrations, and suggests that the majority of HPC bacteria and consequently the majority of airborne micro-organisms aerosolized during land application of biosolids are derived from soil. Further investigations into this phenomenon appear to be warranted.

Although norovirus genomic material was detected upon three occasions via RT-PCR, it is unknown whether these were infectious viruses as no culture system is available for this virus. In addition norovirus nucleic acid was never detected further than 5 m downwind from application sites. However the presence of norovirus RNA suggests that during biosolids operations, it is possible that infectious norovirus may be aerosolized as well.

Liquid biosolids operations yielded levels of indicator bacteria below levels generated by 'cake' biosolids operations. Spray tanker operations did not readily yield concentrations of indicator bacteria or coliphage above detection limits possibly because of the particle size creation. This may be due to spray tankers that generate dense liquid droplets of biosolids that fall to the ground quickly upon aerosolization, limiting the opportunity for transport of aerosolized micro-organisms. However, land application of low solid percentage (2%) liquid biosolids through the use of irrigators generated smaller less dense droplets, leading to detection of *C. perfringens*, total coliforms, and coliphage from distances up to 40 m downwind of the site, although neither was detected frequently. Both processes of liquid biosolids application are rarely used throughout the country based on field observations.

Overall microbial risk of infection to residences near a land application of biosolids site, specifically 'cake' application does exist however it may be within acceptable limits based on this study. As such, boundary minimum limits (30·5 m) appear to be of adequate distance to ensure the safety of nearby public contact with aerosolized micro-organisms associated with land-applied biosolids.

Biosolids spreading operations would appear to present little risk of infection from annual exposure to aerosolized *Salmonella* spp. as biosolids applicators are moving point

sources of aerosols, and little time is spent at one specific location on site. Hence the duration of exposure would be very limited at a fixed location, i.e. a single residence. It is also important to note that for spreading operation risk calculations, aerosolized coliform bacteria detection limits were utilized to estimate aerosolized *Salmonella*. In doing this, samples which were negative for the presence of aerosolized coliforms were assumed to contain minimum detectable limits of coliforms to estimate risk.

Exposures to *Salmonella* spp. during loading operations at downwind distances of 30·5 m proved to be more significant. Annual risks of infection at 30·5 m downwind proved to be greater based on the loading operation. Some assumptions (50% ingestion) used in the risk calculations may not be reflective of how *Salmonella* spp. are transmitted, as there is no reported aerosol transmission to humans for *Salmonella* spp. These risk analyses assume that 50% of all inhaled bacteria are also subsequently swallowed, however bacteria may be associated with particles much larger than 10 $\mu$m and subsequently be expelled via the nose. In addition, loading operations typically are not situations where the duration of residential exposure would be great because of their short duration. However loading situations present the greatest exposure for a residence located directly downwind of these operations as it is a stationary operation (nonmoving point source). It is noteworthy to state that this study does not take into account other pathogenic bacteria present in biosolids and assumes that the greatest risk would be from a pathogenic bacterial genus (*Salmonella*) that is more prevalent in biosolids. In addition, this study assumes that *Salmonella* has a direct correlation to coliform bacteria present in biosolids to assume the pathogen load in the air. Faecal coliform bacteria do have a relationship with *Salmonella* in biosolids, which has been previously demonstrated (Yanko 1988). However this relationship may not be present when considering other pathogenic bacteria and may not be relevant to aerosols.

Annual exposures to coxsackievirus A21 during spreading operations at 30·5 m resulted in a significant risk of infection, when compared to the EPA acceptable standard of 1 infection per 10 000 ($1 \times 10^{-4}$) exposed annual risk of infection from drinking water (Regli *et al.* 1991). It is noteworthy to state that risk of infection calculations from coxsackievirus A21 *vs Salmonella* proved to be greater as the $ID_{50}$ used to calculate risk of infection for coxsackievirus A21 is three orders of magnitude less than that of *Salmonella*. However, the number of *Salmonella* predicted by total coliforms in biosolids compared to the number of human pathogenic viruses in biosolids compensates for this difference, resulting in nearly equivalent risk values. It is also important to state that for spreading operation risk assessment purposes, aerosolized coliphage detection limits were utilized to estimate coxsackievirus A21, and that the same assumptions and overall conservative nature of the risk calculations apply as stated for the *Salmonella* spreading risk calculations. However, the detection limit values were similar to actual detected aerosolized coliphage concentrations during loading operations, hence similar results between spreading and loading risks of infection. The use of coliphage as an indicator of enteric viruses present in biosolids is a source of uncertainty, as to date there is no study showing this relationship exists, however to date an approved indicator for enteric viruses in biosolids is still lacking. As such, the same can be said regarding aerosolized enteric viruses and aerosolized coliphage from biosolids land application.

Exposures during loading operations resulted in the greatest annual viral risks of infection at 30·5 m. These calculations may overestimate the risk of infection from coxsackievirus A21, as the concentrations of coxsackievirus A21 present in biosolids may not be that significant. Conversely these viral risk calculations do not represent risk from other known enteric viruses, such as norovirus, which could be present at greater concentrations in biosolids but require ingestion to initiate infection, hence reducing the likelihood of risk from exposure to aerosols.

A potential source of uncertainty involved in all the risk calculations was in sample collection. Although indicators were rarely detected and with little frequency, the transport modelling approach used the average of detected aerosolized indicators during loading operations and detection limits during spreading conditions to estimate transport of pathogens, rather than the use of averages of all samples collected during these operations. This prevented the use of overly dilute averages, as the majority of samples were negative. As aerosol samples were collected during 20-min sampling periods, it is conceivable that during this sampling period, high concentrations of aerosolized coliforms or coliphage may have been missed. However, the number of samples collected per sampling site (*c.* 40 samples) would appear to reduce this uncertainty. Distribution plots for aerosolized HPC bacteria, total coliforms, and *E. coli* were plotted, and it was determined that among detected concentrations, these groups of microbes were normally distributed at the $\alpha = 0{\cdot}05$ level (data not shown).

A sensitivity analysis in which input factors that influenced daily dose were varied by ±10%. These factors influenced variations in annual risk of infection from exposure to coxsackievirus A21 and *Salmonella* by ±10% (data not shown). Each factor was varied one at a time and included time of exposure, pathogen concentration, breathing rate, and percent ingestion. In addition the number of days exposed annually was varied in a similar manner, and resulted in ±10% variation in annual risk of infection calculations. As demonstrated by the sensitivity analysis, no one exposure factor dominated over the other factors,

hence each carried equal weight within the risk assessment. However, when parameters within the dose response model, such as '$\alpha$', '$r$' and '$N_{50}$' were varied by ±10%, the '$\alpha$' parameter exhibited an increase in risk by five orders of magnitude when decreased by 10%. The '$r$' parameter altered risk calculations by two orders of magnitude when varied by ±10%. These two parameters, which describe the infectious capabilities of the pathogens in question, influenced the risk calculations the most and are the largest source of uncertainty within the calculations.

Overall, residential risks of infection from annual exposures to bioaerosols generated by land application of biosolids proved to be minimal, even at distances of 30·5 m downwind of the source. However the annual risk is present and was calculated to be as high as $4 \times 10^{-4}$ or 0·04% annual chance of infection from exposure to coxsackievirus A21. However, risk of infection posed to the biosolids handler (i.e. occupational exposure) is far greater and can reach as high as 34% annually from exposure to coxsackievirus A21 and 2% annually from exposure to *Salmonella* spp. as reported by Tanner (2004). This is expected as occupational exposure takes place on a daily basis (250 d $year^{-1}$) where as residential exposure is more incidental and passing, typically for only a few days per year. This study presents only risks of infection, while risks of disease could be assumed to be *c.* 10% that of infectious risk calculations although this varies based on micro-organism (Haas *et al.* 1999). It is important to note that while this study assumes risks at 30·5 m to be residential risk, most residential exposures are located at greater distances from application sites. Based on this study, overall residential risk of infection from exposure to aerosols generated by land applied class B biosolids does exist, however it appears to be within acceptable limits.

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