BIOLOGICAL AEROSOLS GENERATED FROM THE LAND APPLICATION OF BIOSOLIDS: MICROBIAL RISK ASSESSMENT

by

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A Dissertation Submitted to the Faculty of the

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2004

The University of Arizona ®
Graduate College

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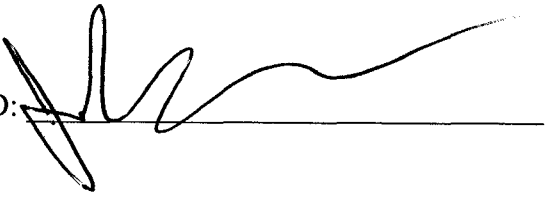
SIGNED: 

ACKNOWLEDGEMENTS

My thanks go out to all who were involved in this study. Specifically I would like to thank Dr. Ian L. Pepper and Dr. Charles P. Gerba, without their guidance throughout these years there would not have been a study to speak of. I would also like to thank Dr. Charles N. Haas, Dr. Raina M. Maier, and Dr. Christopher Rensing for their teachings, assistance, time, and support throughout the years. In addition, Benjamin D. Tanner was a great aid and his hard work throughout the study specifically in sample collection and accompanying me on all trips was greatly appreciated. Karen L. Josephson deserves many thanks for her assistance and aid throughout my laboratory life, none of this would be possible without such a great laboratory manager. In addition I would like to mention Syreeta, and Jessica for their assistance in sample processing.

I would like to thank those who were in the laboratory, as peers but most importantly as dear friends throughout my graduate career: Benjamin Tanner, Scott Stine, Dima Kayed, Terry Gentry, Kathleen Zaleski, Jepson Sutton, Kelly Bright and Jaime Naranjo. I would also like to mention the staff for making my life easier: Kelley Riley, Patricia Orosz-Coghlan, Sheri Maxwell, Pat Gundy, and Elenor Loya.

Finally, last but not least I would like to thank my family: Cecy, Mom, Dad, Tony, and the rest of my family. Without all your support and love none of this could ever have happened.

DEDICATION

I would like to dedicate this to my loving wife, for all her support and love throughout the journey we started years ago.

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ABSTRACT

In the United States greater than 6 million dry tons of biosolids are produced nationwide, with greater than 60% being land applied. Although most counties utilizing land application are practicing this beyond nearby homes, the increase in population has begun to blur the line between rural and urban communities. This study was conducted to investigate the occurence of biological aerosols (bioaerosols) containing microorganisms and endotoxins, and assess the human health risk involved in these practices. Aerosol samples were collected for 2 years from land application sites located at various locations throughout the U.S.A., which represented different climatic conditions and different application practices. Land application practices involved the use of liquid biosolids spray and "cake" biosolids applicators depending on location. Bioaerosols were collected via the use of six SKC Biosamplers, impinging air at a rate of 12.5 L/min for a total of 20 minutes. Samples were collected from both downwind of land application and background sites from distances ranging between 2 m and 70 m downwind. Microbial concentrations were measured within these aerosols, measurements included: heterotrophic plate count bacteria (HPC), coliphage, *Clostridium perfringens*, total coliforms, *Escherichia coli*, endotoxin (lipopolysaccharide), enteroviruses, norovirus, and Hepatitis A virus (HAV). In addition a model was developed to predict viral transport. Overall the levels of aerosolized indicator bacteria and phage were at or below detection limits. Three samples were positive for the presence of norovirus viral RNA via reverse transcriptase polymerase chain reaction, although their viability was unable to be determined based on current available

techniques. Calculated microbial risks of infection were determined to be at or below the acceptable risk of annual infection from drinking water proposed by the Environmental Protection Agency, 1:10,000. Biosolids loading scenarios presented the greatest risk of infection, partly due to the point source of exposure. All other portions of biosolids land application operations yielded risks of infection well below the annual 1:10,000 risk of infection. Overall the microbial aerosol exposures brought about by land applied biosolids are minimal and hence minimal overall risks of infection.

INTRODUCTION

Problem Definition

I. Bioaerosol Exposure from Land Applied Biosolids

Biological aerosol (bioaerosol) exposure is a commonplace occurrence throughout human life. A biological aerosol is an aerosolized biological particle. Whether in the form of a sneeze on a subway car, the natural aerosolization of spores from fungi, or exposure to downwind concentrations of aerosolized bacteria from a wastewater treatment plant, exposure can be found everywhere. While most of these exposures are commonplace, some are not, such as from aerosols from the land application of biosolids. Biosolids are the solid byproduct of wastewater treatment and are treated to reduce concentrations of pathogenic microorganisms. There are two types of biosolids, Class A biosolids are treated to reduce microbial pathogens to levels acceptable enough to be utilized by consumers whereas Class B biosolids on the other hand are treated to reduce pathogenic microorganisms enough to levels acceptable enough to be land-applied to agricultural non-food crop fields. The majority of concern is associated with Class B biosolids as this type is known to contain microbial pathogens such as *Salmonella* and enteric viruses. Exposure to bioaerosols from land applied biosolids have been typically limited to rural America based on field observations, but due to population expansion many Americans are coming into contact with these types of aerosols and subsequently general interest is rising.

Since many communities bordering a land application site are taking interest in the process, in some cases less than 1 mile from their homes, research in this area of

environmental microbiology is also peaking. These communities have gone as far as to protest and even effectively ban biosolids land application in their respective communities. The Environmental Protection Agency (EPA) has set rules (Part 503 rule) designed to protect the community from exposures to biosolids based on limited public access to the site, maximum allowable pathogen concentrations to be applied to the land, and limited food crop use. To date no maximum microbial risk from exposure to bioaerosols from the land application of biosolids has been proposed. Despite the protection afforded to the public from the Part 503 rule, these communities have concerns about this practice.

Literature Review

A literature review entitled: Bioaerosol Emission, Fate, and Transport from Municipal and Animal Wastes is presented within APPENDIX A.

Dissertation Format

This dissertation is presented in a format in which manuscripts, either already published or in the process of manuscript submission, are presented in appendices following this introduction. Appendix A contains a literature review published in the Journal of Residual Sciences and Technology, Appendix B contains a manuscript submitted to the Journal of Applied Microbiology, Appendix C contains a manuscript formatted for the Journal of Applied Microbiology, and Appendix D contains a manuscript formatted for the Journal of Applied Microbiology. The research was all conducted at the University of Arizona in the laboratories of Dr's Ian L. Pepper and Charles P. Gerba. All research and published work was conducted and written primarily by myself. In addition Benjamin D. Tanner assisted in collection of samples and editing of manuscripts. Karen L. Josephson provided invaluable support in the laboratory and assisted in the processing of aerosol samples. Dr. Charles N. Haas assisted in editing of the manuscript and provided support in calculation of risk. Dr. Charles P. Gerba assisted in editing of the manuscript and provided invaluable research direction. Dr. Ian L. Pepper was involved in all planning of research, direction of research, and finally provided invaluable aid in editing all manuscripts.

PRESENT STUDY

The methods, results, and conclusions of this study are presented in the papers appended to this dissertation. The following is a summary of the most important findings in these papers. The work presented in this dissertation was conducted over a period of 2 years, in which bioaerosol samples were collected from numerous sites throughout the continental United States. Sites included: Marana, Az, Eloy, Az, Picacho, Az, Mojave, Az, Solano, Ca, Snoqualmie, Wa, Sunnyside, Wa, Leesburg, Va, Houston, Tx, and Chicago, Il. Air samples were collected via the use of six SKC Biosamplers® all located downwind of the land application of biosolids. Samples were collected from operations involving biosolids loading, unloading, application, and background samples. Following sample collection, all were analyzed for the presence of pathogenic indicators and pathogenic microorganisms including: Heterotrophic Plate Count bacteria (HPC), total coliforms, *Escherichia coli*, *Clostridium perfringens*, coliphage, and enteroviruses, Hepatitis A virus, norovirus. Cultural techniques were used to determine aerosol concentrations of indicator bacteria and coliphage, while molecular techniques such as reverse transcriptase polymerase chain reaction (RT-PCR) were used for the detection of the viral RNA genomes. In addition aerosolized endotoxin concentrations were also determined from sites located throughout southeast Arizona. An empirically derived transport model was developed from the transport of coliphage from aerosolized water and subsequently used to model viral transport. Overall it was found that minimal risk from microbial aerosol exposure exists. Determined aerosolized microbial indicators were used to model the aerosolization and transport of their pathogen counterparts from

biosolids loading and application scenarios, both liquid and solid biosolids. Loading scenarios presented the greatest risk although most communities do not come into contact with this operation. Endotoxin exposures were found to be below suggested levels for occupational exposures such as compost plants, although the detected concentrations were within ranges known to cause both acute and chronic lung effects. All other aspects of the operation presented risks far below the suggested 1:10000 annual risk of infection from exposure to microbial pathogens present in drinking water. At present time there is no suggested annual risk of infection maximum from aerosol exposures.

APPENDIX A

BIOAEROSOL EMISSION, FATE, AND TRANSPORT FROM MUNICIPAL AND ANIMAL WASTES[1]

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Published, 2004 Journal of Residual Science & Technology *1:15-28*

Abstract

This review concerns the generation and fate of bioaerosols generated from the treatment of wastewater, composting plants, and during handling and land application of wastewater and biosolids. Though many bioaerosol studies have been conducted on composting and wastewater treatment plants, few studies have been conducted on land-applied biosolids. Wastewater treatment and composting plants generate almost a constant source of aerosols during plant operation, but bioaerosols tend to be contained within the plants and pose the greatest risk towards the workers themselves. Land application sites, whether wastewater application or biosolids application, are of concern as communities are beginning interface with rural areas where land application occurs. However, the majority of the available data, suggests that land application operations pose little risk towards the general public with respect to infection from bioaerosols. Aerosolized microorganisms generated by any of these land application operations appear to be inactivated relatively quickly as many are already in stressed physiological states, and the aerosol environment is also a harsh environment. Inactivation can occur via environmental dessication, ultra violet light, and oxygen radicals. In the Dowd et al., paper (2000) "worst case" scenarios during land application of biosolids predicted a risk of infection of 1.00 (100%). However an incorrect infectivity constant (r) was used in this calculation. Using the correct (r) value and more realistic values of phage:human virus ratios, the predicted risk is 5 orders of magnitude less than 1.00. In recent years biosolid treatment has improved resulting in lower pathogen concentrations, and even less potential for aerosolization. Risk that does exist can be reduced for waste-treatment workers through the use of hygienic practices, and towards the general public via the implementation of appropriate buffer zones. Overall, the risk of infection via a bioaerosol of land applied biosolid origin is low.

Keywords: bioaerosol, biosolids, sludge, pathogen, risk

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Introduction

Through the reuse of municipal waste, in the form of wastewater, biosolids land application, and composting of sewage sludge, there exists the potential for transfer of pathogens as bioaerosols from the operation site to surrounding communities [40,51]. In addition bioaerosols can also be generated through the operation of wastewater treatment plants or composting plants, both of which can be found within city limits or within a few hundred meters of homes. Despite the potential for bioaerosol generation from these operations, the risk of infection to the general public has not been well documented [40]. Bioaerosols consist of microorganisms or other biological particles such as endotoxin or peptidoglycan that become airborne, with the potential to be transported over significant lateral distances. If the microbes transported are pathogenic, then exposure to them potentially becomes a human health issue. Recently, the potential for aerosolization of pathogens from land application of biosolids has become an issue that has been debated nationally. To date, few studies on land application of biosolids have been conducted, but several studies have evaluated aerosols from wastewater treatment plants, land application of wastewater, animal manures, and composting operations. Overall, the potential for adverse health effects from pathogens in bioaerosols depends on their fate and transport. The fate, and inactivation of aerosolized microbes is affected by numerous environmental factors and methods of aerosol generation, while transport, or the lateral distance bioaerosols are carried from source to endpoint, is affected by factors such as wind direction and velocity [28,38]. Despite the generation of bioaerosols, if the

microbes contained within, are either inactivated or fail to be transported over any significant distance, is there actually a risk? This is the fundamental question that requires answer. Risk from these operations is typically thought of as being highest amongst workers that handle the waste material, but community interest in the potential for bioaerosols has recently been increasing. This review will focus on available studies and data on bioaerosols generated from wastewater treatment plants, wastewater land application, biosolids land application, and composting sites.

Characteristics of Bioaerosols

The term bioaerosol is used to describe biological particles, which have been aerosolized [28]. These particles may contain microorganisms (bacteria, fungi, viruses) or biological remnants such as endotoxin and cell wall constituents such as peptidoglycan [28]. Bioaerosol sizes range typically from 0.5 to 30 μm in diameter and are typically surrounded by a thin layer of water [49]. In other instances, the biological particles can be associated with particulate matter such as soil or biosolids, depending on the place of origin [34]. Bioaerosol particles in the lower spectrum of sizes (0.5 to 5 μm) are typically of most concern as these particles are more readily inhaled or swallowed [49].

Bioaerosols generated from the land application of biosolids may be associated with soil or vegetation depending on the type of land application. For example, if a front-end loader is used to load the biosolids spreader, it is possible that soil will be in contact with the biosolids, and therefore be associated with any bioaerosol generated by it. In this situation the soil particle or vegetation is known as a "raft" for the biological particles

contained with the bioaerosol [34]. However, for soil particles to be aerosolized, the particles need to be fairly dry, and low soil moisture contents are known to promote microbial inactivation [50].

Methods for Bioaerosol Collection

Critical to assessing the generation of bioaerosols is the type of sampling employed. Currently there are two main approaches that have been utilized to study bioaerosols: surface impaction; and liquid impingement [2,11]. Regardless of which method is utilized, sampling is routinely done at a height of around 1.5 m above ground level corresponding to the average human breathing height [2,18]. Normally a downwind sample is collected at a distance of between 2 and 500 m from a target point source. Typical standard sampling distances are 2, 15, and 50 m downwind, that are subsequently used to create a linear regression relating aerosol concentrations to specific distances from the point or area source [2,20]. In most studies, samples have been collected within 50 m of the source and frequently within 20 m. In addition, an upwind (background) sample from the source is also taken to account for the normal ambient microbial air densities [2]. Samples are collected during suggested meteorological conditions that include a maximum wind speed of 6.7 m/s, and a wind direction change of less than 90 degrees within 15 minutes, although samples are collected during conditions that do not match these requirements [5]. Of these two, wind direction change is of most importance, since as wind changes direction, the direction of the bioaerosol plume may not be accurately represented in a downwind or an upwind sample. The entire sample

collection process may be as short as a few minutes, or as long as 8 hours, depending on the sampler used and specific parameters being measured. For example, when sampling for enteric viruses or other microbes, which may be present in low aerial concentrations, it may be necessary to sample a large volume of air [39]. Advantages of using large volume samplers include, increased volume of air from 0.25 cubic meters of total air sampled using an impinger, to 1.5 cubic meters of air per minute using high volume electrostatic precipitators, although microbial inactivation may increase. Alternatively sampling precision and volume sampled can be increased through the use of multiple samplers used in an array with simultaneous measurements at discrete locations.

Bioaerosol Sampling via Liquid Impingement

Liquid impingement typically involves collection of an air sample into a buffered liquid trapping agent such as water amended with 0.1% peptone. Air and biological particles are drawn through a single glass inlet depositing the bioaerosols into a solution through inertial forces, which remove the particles from the air [11]. This solution allows particle movement as the liquid is agitated during the sampling process, thus breaking apart any cell aggregates, and also allowing for a gentler impaction than that found with surface impaction. Survival of microbes is greater with liquid impingement than with solid impaction. The ability to collect microorganisms within a liquid also allows for a greater variety of microbial detection methodologies, including culturable assays as well as molecular methods such as Polymerase Chain Reaction (PCR) or Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) [1,3,47]. Culturable methods are simple to perform and

extremely common, but it has been shown that many bacteria remain viable but lose their ability to grow and form colonies on culturable plates, due to the aerosolization process or during the collection process [15,30]. However, this limitation can be overcome by using microscopic techniques and stains that differentiate viable organisms [30]. Molecular methods such as PCR are very sensitive since the technique detects nucleic acid sequences associated with specific pathogens. However, a positive PCR result does not necessarily indicate viability [32]. The AGI-30 (Ace Glass Inc., Vineland, NJ) was originally intended to be the unit of choice when collecting samples utilizing the impingement method, but variations of this device are also commonly used. Evaporation of the liquid buffer tends to be a problem particularly when sampling for more than 20 minutes, but this can be alleviated by using impingers such as the SKC Biosampler® (SKC West Inc., Fullerton, CA) in combination with mineral oil [35]. Mineral oil allows the collection of a sample for a longer period of time, and the detection of microbes present at lower aerial concentrations. Typical collection times are 15-30 minutes for water based buffers, and up to 8 hours for oil based buffers [35].

Bioaerosol Sampling via Surface Impaction

Surface impaction is similar to impingement except a solid surface such as an agar plate is used to collect the sample. Most commonly used systems for surface impaction are the SAS 100® (Surface Air System) (Bioscience International, Rockville, MD) or the Anderson 6 stage sampler® (Anderson Instruments Inc., Smyrna, GA). The SAS system works by drawing air in through a perforated surface, and utilizing inertial forces to

deposit air particles onto an agar medium [11]. The Anderson 6 stage sampler is similar in theory, except that particles are deposited onto successive stages that aid in determining the size of particles. Heavier particles deposit onto the first stage, and lighter particles bounce off this stage and travel via air currents onto successive stages. These systems allow for the direct cultivation of bacteria and fungi onto the agar surface. Although this is convenient, it does inhibit the user from utilizing multiple types of assays. If other assay methods are desired the organisms can be washed off the surface of the collection plate by a liquid buffer [44]. A major advantage of using these systems is that large volumes of air can be sampled within a short period of time for example the SAS 100®, can collect samples at the rate of 100 L/min [11].

Factors Affecting the Fate of Microorganisms within Bioaerosols

Several parameters influence the fate and viability of bioaerosols in the environment. Physical characteristics of the bioaerosol and environmental factors are primary parameters involved in the survival of microbes within a bioaerosol. Size, shape, chemical composition, and density of the bioaerosol strongly influence fate as well as transport [38]. Environmental factors including atmospheric conditions also affect fate and transport (Table 1) [38]. Relative humidity has long been recognized as one of the most important factors involved in bioaerosol viability, and has been evaluated in laboratory studies that were able to isolate relative humidity as a single variable. Under laboratory conditions aerosolized cells of the Gram-negative *Escherichia coli* bacterium have been shown to exhibit almost 100 percent survival during conditions of low to mid

levels of relative humidity, with enhanced decay at relative humidity above 80 percent [16]. The opposite is true for Gram-positive bacteria, which exhibit decay at low relative humidities [56]. In similar fashion, viruses containing a lipid envelope demonstrate increased inactivation at high relative humidity, where as naked capsid viruses exhibit increased inactivation at low relative humidity [38].

Bacterial inactivation through dehydration and desiccation processes occurs as relative humidity decreases and temperatures increases. This results from conformational changes in the phospholipid bilayer of the microbial cell wall due to a lack of cell available water [31]. In general, Gram-negative bacteria react unfavorably to desiccating conditions whereas Gram-positive cells, are more able to withstand desiccation stress [36,38]. Temperature is also known to play a significant role in microbial survival in aerosols. The effects of temperature are difficult to isolate from the effects of humidity as the two are frequently intertwined [38]. Overall, greater temperatures tend to favor microbial inactivation [17]. Bacterial membrane phospholipids and proteins are the main targets of temperature induced inactivation [38]. Viruses, which lack these membrane components tend to be more resistant to effects of temperature induced inactivation [38]. Lipid containing viruses tend to be more stable at low relative humidity, but the effects of temperature alone, are not as critical to virus survival [38]. Oxygen concentration, another important factor in microbial survival is involved in inactivation of bioaerosols through the production of oxygen free radicals [28,38]. The effects of dessication are further enhanced by oxygen radicals that when combined with dessication, are thought to

contribute to the inactivation of microbes [31]. Ultraviolet radiation can also detrimentally affect bioaerosols, with bacteria once again being more susceptible [38]. Ultraviolet rays damage DNA by forming thymine dimers; which prevents the cell from dividing and reproducing. Wind speed and direction correlate with overall transport of bioaerosols and may or may not affect viability, although this has not been well studied. Overall bacteria tend to be less stable in the aerosolized state than viruses, with the exception of spore forming bacteria, such as *Clostridium* spp [38].

Transport of Bioaerosols

Because of the rapid dilution of aerosolized microorganisms, transport models are necessary to predict viable concentrations at distances of interest from the source of generation. Models are useful to predict the fate of pathogens which can not easily be measured in aerosolized form because of low concentrations in the aerosol or lack of methods for their detection. There are three important factors needed to model microbial fate: I) release or emission from the source; II) dispersion; and III) deposition [38]. Release involves the particle's ability to break away from the source material such as liquid biosolids. Environmental forces such as wind can provide the energy to initiate the emission of a bioaerosol [27,38]. Mechanical forces can also be provided in the form of agitation of the source material such as in the mechanical agitation of wastewater, mechanical agitation of biosolids, or human activity [10,14,18]. Energy to allow release of viruses from biosolids is particularly important since studies have shown that viruses are sorbed or embedded within biosolids and not easily released for subsequent transport

[6]. Once a particle is released from its source material, the particle is subject to transport via prevailing air currents, convection, diffusion, and gravitational settling. Smaller particles below 5 μm are transported via air currents, while larger particles tend to leave the air currents and deposit onto surfaces. Other methods of particle movement include convection via temperature variations, and diffusion via concentration gradients [21]. Deposition is the actual settling of the particle and is controlled by the mass and density of the particle. Deposition onto a surface, once a particle is within the vicinity of a surface, can be controlled by low energy bonds such as Van der waals forces, and electrostatic forces, referred to as adhesion forces [38].

Models that have been commonly involved in predicting transport in the past have been based on aerosolized inert particles from either a point source or an area source. These models take into consideration release from source material, transport via air currents, and plume distribution making for a complex equation with multiple variables. The models were originally used to demonstrate the fate of air pollutants, and are limited to constant wind speeds under conditions where flat terrain is prevalent [42]. Under actual outdoor conditions this may not always be the case as wind gusts, and periods of no wind will greatly influence how and where a bioaerosol is transported. Historically most models have not taken into account microbial decay since the models were primarily designed for modeling inert particle dispersion [38]. Realistically, microbial decay must be applied to accurately predict the fate and transport of viable bioaerosols. Since different microorganisms react differently to each set of environmental parameters,

microbial decay coefficients need to be calculated for each microorganism under a specific set of environmental conditions. Decay (die-off) constants (Table 2) are used to predict how quickly a viable bioaerosol will be adversely affected during its travel time, be it seconds or minutes [38]. These constants help in predicting how far a viable aerosolized microorganism can be transported.

Bioaerosols from Wastewater Treatment Plants

Bioaerosol emissions from wastewater treatment plants (WWTP) have been evaluated in several studies due to concern of exposure to surrounding neighborhood and WWTP workers. Most studies tend to agree that the potential for bioaerosol formation does in fact exist, but the significance of this problem is still disputed. In a study conducted by Carducci et al. (2000), it was stated that indoor sewage washing stations contained the highest amount of airborne bacterial and viral contaminants, thus posing the greatest risk towards WWTP workers [14]. Significant sources of bacterial aerial contamination were also detected in areas where the wastewater was mechanically agitated (i.e., mechanical aeration basins). It was found that aerosols contained non-pathogenic intestinal bacteria such as coliform bacteria, but some enteric pathogens were isolated including *Salmonella enteritidis* and *Shigella boydii* [14]. It is noteworthy that these organisms have not been shown to be transmitted via inhalation, but can be transmitted through deposition on commonly touched areas in the plant such as stair rails and other inanimate objects, subsequently allowing for the fecal oral route of transmission [14].

Carducci et al. also noted that coxsackievirus B and reovirus were also recovered,

which was of concern to the investigators, since these viruses do present a risk from a respiratory route of infection [14]. It was determined by the authors that WWTP workers would inhale at least 2 virus particles per 8 h work day when there was at least a 1 virus per 3 m^3 aerial concentration [14]. Reoviruses were consistently found to be present when other enteric viruses were present in this study, and were suggested as a potential indicator of aerosolized enteric viruses [14]. Fecal streptococci and coliphage have previously been thought of as being suitable indicators of WWTP bioaerosols, as they were found to be resistant to environmental stresses such as desiccation, heat, and ultraviolet rays [13,18]. However, the results of a study by Carducci et al. (1999) noted that coliphage, while being an adequate indicator of enteric virus behavior in the environment, had no correlation when used as an indicator of aerial enteric viral contamination. In addition total bacteria and fecal streptococci ($P < .05$) had a significant correlation with aerial viral contamination [13]. Carducci's study has shown that enteric viral aerial contaminants could survive longer than traditional indicators, such as coliphage, coliforms, and fecal streptococci. Concentrations of enteric viruses such as, reovirus and enterovirus decreased by 15% at 50 m, whereas all other indicators decreased by more than 88%, with coliphage decreasing by 99% [13].

In a study by Brandi et al. (2000), aeration basins yielded few significant concentrations of aerosols even though they were believed to be significant sources of bioaerosols. This was believed to be due to the differences in types of aeration basins where mechanically agitated aerators yield aerosols and diffuse oxygenation systems

yield little or no aerosols above normal ambient levels as shown in Table 3 [8]. When aerosols were created by the aeration basins they were found to contain staphylococci, coliforms, *Escherichia coli*, and enteroviruses. However, none of the staphylococci were confirmed to be *Staphylococcus aureus*. Specifically, *Staphylococcus* spp., coliforms, *E. coli*, and enterococci were found 20 m downwind of the tank, and the authors felt this posed a significant potential for aerosol exposure to WWTP workers [8]. Along with aeration basins, trickling filters have also been thought of as significant potential sources of aerosols.

This was found to be the case in a study conducted by Goff et al. (1973), who found that as wind travels over a wastewater treatment plant trickling filter, its total bacterial, and total coliform concentrations increased [27]. Multiple meteorological factors were found to directly impact the viability of microbes found within bioaerosols. Windspeed and relative humidity factors when analyzed together were found to be important factors in relating to aerosolized microorganisms' viability [27]. A median wind speed of about 6-10 mi/hr, combined with greater than 35% relative humidity resulted in a greater aerosol emission with greater microbial survival, as shown by greater concentrations of coliforms bacteria as relative humidity increased [27]. Deviations of windspeed, either greater or lower speeds, yielded lower aerosol emissions. Solar radiation has been shown to be a contributing factor amongst aerosols generated by WWTP, as night-time coliform and total bacterial numbers increased significantly, by at least 1 $\log_{10}/m^3$ of air as compared to afternoon samples near wastewater treatment plants [24,27].

Bioaerosols Generated through Land Application of Wastewater

Although land application of wastewater could potentially result in greater creation of bioaerosols, depending on the method of application, it is less well studied than WWTP. Wastewater can be applied to land via three general methods [26]. Wastewater can be utilized via irrigation, in which sewage effluents are applied to land through the use of sprinkling systems at a low-rate of application. The overland flow system, allows the effluent to be sprayed over a field where the effluent, following a lateral travel distance, is collected and pooled into a collection ditch [26]. As the effluent in both these scenarios is applied via a sprinkler system, both these methods are condusive to aerosolization, whereas in high rate infiltration, effluent is percolated through the soil [26].

One study by Teltsch et al. (1977) involving wastewater spray irrigation found that when a bacterial concentration of greater than 10^3 cfu/ml in wastewater occurred, there was the likelihood of detectable bioaerosols [53]. In the same study, night-time irrigation resulted in aerosols that were found to contain at least a one $\log_{10}$ increase in bacterial concentrations as compared to daytime irrigation [53]. This was due to lower overall temperature, higher relative humidity, less solar irradiation, and overall more stable atmospheric conditions. The authors also stated that irrigation often occurs at night enhancing the likelihood of microbial survival in bioaerosols [53]. Wind speed appears to play a less significant role in land application as compared to WWTP studies, where wind speed has been shown to play a significant role in aerosol production. This may be due to the fact that these aerosols are already launched from their respective point sources

by irrigation processes or spray-gun processes, whereas at a WWTP, the aerosol particle almost inevitably needs wind or another type of mechanical agitation to aid in the initial transport of the particles from the point source.

In a study conducted by Teltsch et al. (1980), pathogenic bacteria and viruses were identified in aerosols near a wastewater irrigation site, utilizing multiple sprinklers with varying effluent discharges of 1.7, 4.5, and 100 m^3/h [54]. The focus of this study was *Salmonella* and enteroviruses, which were detected at low levels in the air, despite concentrations in the effluent of between non-detection and 60 MPN/100ml, and between non-detection and 4 $\log_{10}$ PFU/L, respectively [54]. *Salmonella* sp. were able to survive in air for longer periods than coliforms, and the authors were quick to state that coliforms did not fulfill one of the main criteria of indicator organisms, this being longer survival in the environment than the pathogen in question. Although coliforms were detected in every air sample collected, they were present at concentrations less than *Salmonella* [54]. Of the identified enteroviruses: poliovirus, echovirus, and coxsackievirus B were the most prevalent, and were detectable over 100 m downwind of the point source [54]. As the distance from the site increased from 43 m to 100 m downwind, the ratio of enteroviruses to coliforms increased by about one $\log_{10}$ indicating less inactivation of aerosolized enterovirus than coliforms. At distances greater than 100 m, coliforms were no longer detected, whereas enteroviruses were still found, indicating that coliforms had increased susceptibility to inactivation during transport. This was further demonstrated in another study by Teltsch et al. (1980), where *Escherichia coli* concentrations decreased

by ninety percent within the first ten seconds of aerosolization during the afternoon. In contrast, reduction rates in the morning, demonstrated a 90% reduction within the first 100 seconds of aerosolization [55]. This was attributed to the harsher ambient weather conditions present in the afternoon, including relatively low humidity and increased solar radiation.

Camann et al. (1988) found significantly elevated microbial aerial densities at distances greater than 100 m downwind from a wastewater slow-rate irrigation site, that did not decrease until distances were greater than 200 m from the source (Table 4) [12]. It is important to note that the wastewater in use, was untreated with levels of fecal coliforms exceeding 6 $\log_{10}$ per 100 ml and enteric virus levels ranging from 100 to 1000 PFU/L prior to impoundment in a reservoir. The reservoir would reduce levels of coliforms by as much as 99% and viral levels to below 10 PFU/L, it was this wastewater that was aerosolized [12]. Even though concentrations of bioaerosols receded to background levels, the presence of wastewater generated aerosols can potentially be detected through the use of aerosol size determinations [5].

In a study conducted by Bausum et al. (1983), downwind aerosols differed from ambient aerosols not only in composition but also in size. The downwind wastewater-associated bioaerosols were smaller in average size, 2.44 - 3.03 μm versus ambient bioaerosols, 4.15 - 4.59 μm [5]. These differences can aid in the source identification of aerosol contamination. Even at increased downwind distances (>200 m), aerosolized HPC numbered near background levels. However, the aerosol droplet size distribution

was consistent with wastewater-associated aerosols when compared to upwind aerosols thus allowing the authors to conclude that these aerosols were of wastewater origin. Hence an apparent "washout" of ambient microbes had occurred at these distances, where the wastewater-associated bioaerosols would temporarily take the place of the ambient aerosolized microorganisms [5].

Chlorination and long-term storage of wastewater can reduce microbial concentrations thus reducing bioaerosol potential. While chlorination of wastewater is effective in reducing enteric bacteria in bioaerosols, chlorine is less effective on enteric viruses, which are more resistant [4,57]. A study conducted by Bausum et al. (1982) demonstrated that while chlorination did reduce downwind aerosolized bacterial concentrations to near background levels, coliphage was still detected at distances of 137 m downwind [4]. Long-term storage of wastewater involves the storage of the wastewater effluent in a holding tank for at least 30 days, removing up to 99% of the enteric viruses, and thus reducing potential aerosolized viruses [12, 57]. In addition to these two approaches, buffer zones have been found to be a cost effective approach to reducing exposure to bioaerosols. Buffer zones work by providing enough distance to be placed between the spray site and the nearest neighboring residences. These zones vary nationally and can be 65 - 300 m from the aerosol source, thus increasing the cost of wastewater application depending on the value of the land [57].

Bioaerosols Generated via Land Application of Biosolids and Animal Slurries

Recent increases in the extent of land application of biosolids nationally have resulted

in an increased focus on the generation of bioaerosols produced during this process. Since the early 1980's, the amount of biosolids land applied has increased from 20% to greater than 60% of nearly 6 million dry tons applied today nationally [40,41]. In 1999, 94% of Arizona's total biosolids were land applied, and in Southern California this number exceeded 75% (unpublished data). Most land application is on agricultural land allowing nutrients found in the biosolids to be used in a beneficial manner. However, there has been increasing concern among communities and adjacent farms on the safety of this practice partially with respect to the potential for bioaerosols [40].

The bioaerosols generated depend, as in wastewater irrigation, on the method employed to land apply the biosolids (Table 5, Figs. 1-4). Multiple methods do exist, such as the spray gun method (which is similar to the wastewater spray gun), that launches low solid content liquid biosolids into the air hundreds of feet [7]. This method is thought to create the largest amount of bioaerosols, as the launching will most likely disturb the biosolids enough to create the potential for aerosolized microbes [7]. Although this method of application, more recently, is limited in its use, animal wastes have been land applied utilizing this method [7]. The spray of pig slurry from this type of applicator aerosolized total bacterial concentrations between 400 and 2300 cfu/m^3 at downwind distances of between 120-150 m (this was typically about 60 m away from the slurried area) from the source. Total coliforms, fecal coliforms, and fecal streptococci concentrations between non-detection levels to no more than 69 cfu/m^3 at downwind distances of between 70 and 170 m from the source were detected [7]. Fecal streptococci

were found more frequently than fecal coliforms, but overall fecal bacteria were found infrequently in aerosols. Droplet size was generally large, with an average size of > 8-10 μm. Typically a diameter of <5 μm is necessary for effective inhalation by a human being, however diameters of <2 μm deposit into the respiratory system most effectively [49]. The results of the Boutin et al. (1988) study suggested that the usage of reel-spraying guns yielded greater concentrations of downwind bacterial counts when compared to tank spreading.

A study conducted by Sorber et al. (1984) demonstrated a similar result, comparing the operation of tank spreading and high-pressure spray guns [48]. In that study anaerobic digested primary biosolids were applied by spray guns. It was this application method that allowed for the detection of total and fecal coliforms, coliphage, fecal streptococci, and mycobacteria at distances up to 50 m downwind, with a 10-fold increase over upwind levels, which were below detection limits, of total and fecal coliforms, coliphage, fecal streptococci, and mycobacteria [48].

Today spray tankers are a common way to land apply liquid biosolids (Fig. 3). Sorber et al. studied the generation of microbiological aerosols created by tank truck sites. This method allows the minimal amount of dispersion of bioaerosols over the biosolids applied area compared to using a spray gun, and reduces the probability of aerosolizing pathogens. The tanker truck spreads the liquid biosolids close to ground level, at a height of 0.9 - 1.5 m, thus minimizing the aerosol dispersion effect [7,48]. When sampling the tank truck sites, standard plate count bacteria, total coliforms, and fecal streptococci were

indicative of some aerosolization. Standard plate counts were around one and two $\log_{10}$ units above upwind samples, and fecal streptococci/total coliforms were about one $\log_{10}$ above upwind samples, demonstrating a small amount of aerosol originating from the biosolids [48]. The low numbers are attributed to the minimal height above ground level that the tank sprays, thus minimizing the dispersion factor of the bioaerosol. In addition, sampling along a moving point source (tanker truck) proved to be difficult for the authors. In this situation, they decided to place two trios of air samplers 30 to 40 m apart from each other downwind of the truck as close as possible to the truck to create a sampling array. This enabled the samplers to assess the tanker emissions as they passed by each sampler. However, in this scenario, in effect there was only around 2-3 minutes of actual downwind sampling, with the remaining sampling time being equivalent to background sampling [48]. Sorber et al, concludes the study stating that no viruses were detected, even during a sampling event in which air samples were pooled together yielding a 1470 m^3 sample, which was assayed via cell culture. Despite the presence of enteroviruses in the biosolids at mean concentrations of 1-2 pfu/g, the authors implied that "aerosolization of viruses was not a significant problem" [48]. Overall, this study reported that: "In general, microbiological aerosols generated in the application of sludge to land as described in this study do not seem to represent a serious threat to human health for individuals located more than 100 m downwind of the sludge application site. In fact, the data suggest that microbiological concentrations of aerosols are significantly less than those at wastewater spray application sites and to date, no conclusive evidence

has demonstrated an adverse relationship between aerosolized wastewater and human health." [48].

Thickened biosolids can be land applied through the usage of hopper spreader application [18,19,20,43]. A study conducted in Sierra Blanca, TX monitored *Salmonella spp., Clostridium spp.,* coliphage, hydrogen sulfide producing bacteria, and typical indicator organisms (fecal coliforms, fecal streptococci) in bioaerosols [18,19,43]. This method of anaerobic digested class B biosolids application consists of loading the biosolids using a front-end loader onto a biosolids spreader, known as a hopper, with subsequent application to land. The greatest levels of aerosol contamination occurred during this loading operation [18,19,43]. At the loading sites, heterotrophic bacteria (HPC) averaged 4.5 x 10^6 cfu/m^3 and fecal streptococci, *Salmonella* spp., F^+ coliphage, H_2S producing bacteria, and *Clostridium spp.* averaged between 2 $\log_{10}$ and 3 $\log_{10}$ cfu/m^3 [18]. Background levels were between 10 to 100 times less for HPC bacteria, and non-detectable for the enteric bacteria and coliphage. The application site did not routinely produce high numbers of aerosolized microorganisms when compared to the loading site. Typical numbers at the application sites were about 10 times less HPC bacteria when compared to loading sites, exhibiting an average heterotrophic plate count of 1.4 x 10^5 cfu/m^3, and between 1 $\log_{10}$ and 2 $\log_{10}$ cfu/m^3 for other parameters with the exception of fecal streptococci and H_2S producing bacteria, which were below detection. Interestingly, despite the lack of fecal streptococci detection, both fecal and total coliforms were detected with an average of 25 MPN/m^3 at the application site, but were not detectable at

the loading site [18]. This could be attributed to the poor viability that coliforms exhibit while aerosolized, and the randomization of aerosol sampling. The authors conclude the study by stating that perhaps thermotolerant *Clostridium spp.* may be a more reliable indicator of aerosolized enteric pathogens, and that coliforms and fecal coliforms are less reliable [18].

Treatment which biosolids receive (i.e. anaerobic digestion, lime treatment, etc), and overall stresses that aerosolized organisms are placed under suggest that thermotolerant spore forming clostridia would be the most logical choice as an indicator of aerosolized pathogens, but the prevalence of *Clostridium perfringens* within bioaerosols needs additional study. *Clostridium perfringens* as an indicator is also supported by a study by the same authors who found that *Clostridium perfringens* could be ribotyped using the 16s-23s interspacer ribosomal region, and that sources of aerial pollution could be identified according to this DNA fingerprint [19].

A recent study evaluated the presence of *Staphylococcus aureus* in various types and classes of biosolids and sewage sludge across the United States. *S. aureus* could be detected in sewage, but was never detected in Class A or B biosolids. In addition *S. aureus* was not detected in aerosol samples collected from land application sites in Arizona, and California, although different types of biosolids (liquid and "cake") were applied and via different methods of application (liquid spray, and manure spreader) [46]. More recently, an ongoing study evaluating bioaerosols from various methods of land application of biosolids (liquid spray, spreading via manure spreader, and slinger

application) across the continental United States, demonstrated low percentages, (<10% of all samples collected), of positive bioaerosols containing indicators such as total coliforms, coliphage, *C. perfringens*, and *E. coli* [52]. In addition, enteric viruses were rarely found in bioaerosols, and never further than 5 m from the site of application [unpublished data].

Bioaerosols from Composting Sites

In contrast to bioaerosols from the land application of biosolids, many studies have been conducted on composting sites. These studies have focused on aerosolized *Aspergillus fumigatus,*, an opportunistic pathogen, and on endotoxin, the lipopolysaccaride component of Gram-negative bacteria [22,23,25,33,37]. In addition to these parameters, Gram-negative bacteria, total bacteria, thermotolerant actinomycetes, and immunological markers specific to these microbes have also been investigated [9,37].

A more recent review of the literature conducted by Epstein et al (1994) concluded that the majority of aerosolized *A. fumigatus* are confined to within the composting site with off-site levels of *A. fumigatus* reaching background levels [22]. They concluded that even during mixing conditions (operations that involve the mechanical mixing of sludge and wood chips), the levels of *A. fumigatus* were about 1 $\log_{10}$ above that of background concentrations. Background concentrations were found to be between non-detection levels and 1 $\log_{10}$ per cubic meter. The review also noted that to date, no endotoxin levels surrounding composting sites have had negative effects on the surrounding neighborhoods [22]. The authors note that most detected levels of endotoxin were below

the suggested safe level of 0.1 $\mu g/m^3$. The study concluded by stating that the majority of aerosolized *A. fumigatus* occurred during mixing conditions, or when the compost mixture was mechanically agitated, and that these concentrations despite being greater than background concentrations posed little risk [22].

Other studies have shown similar results with regards to *A. fumigatus*, specifically with regards to mechanical agitation of the compost piles [25,33]. Kothary et al (1984) concluded that compost agitation would lead to increased levels of the fungal spores at distances within 50 m downwind of the compost site. Rainfall events would lead to 1 to 2 $\log_{10}$ lower levels of *A. fumigatus* within 50 m of the compost site [33]. In residential areas surrounding composting sites, the aerosol levels of *A. fumigatus* were below 50 CFU/m^3, where as *A. fumigatus* levels at control sites ranged from 0 to 2 CFU/m^3 [33].

More recent work has focused on immunological markers and health complaints of compost workers. The results of a 2000 study conducted by Bunger et al, noted that compost workers had more symptoms and diseases of the airways and skin than control subjects [9]. Increased IgG (immunoglobulin G) antibody concentrations amongst these same workers correlated to the increased exposure to fungi and actinomycetes present in compost-associated bioaerosols [9]. The study also compared the relative exposures amongst biowaste collectors and compost workers. Biowaste collectors were found to have fungal, and actinomycete antibody titres similar to that of control subjects, and this correlated the relative amount of aerosol exposure to these types of microorganisms was correlated to their respective job settings [9]. Exposure to total bacteria, actinomycetes,

and fungal spores increased by at least 1 $\log_{10}$ at composting plants when compared to biowaste collection sites [9].

The Risk Assessment Approach to Assess Health Effects of Bioaerosols

The use of risk assessment models is currently the best method to estimate the risk of infection from exposure to any of these methods of aerosolization [29]. As an example of microbial risk assessment, the following calculations were made from data obtained from Dowd et al [20]. The utilization of mathematical modeling as an approach to microbial risk assessment was new and innovative, as shown by Dowd et al, but the values used to estimate risk particularly viral risk, overestimated the actual risk.

As an example of the risk assessment process, the risk calculations conducted in the 2000 Dowd et al paper will be recalculated using concentrations of human viruses in biosolids more suitable to current reported values. Values used in the Dowd et al paper ranged from 0.2 to 200 PFU/g for human enteric viruses and 10^4/g of phage, whereas current reported values in Class B biosolids are near 0.2 PFU/g, and values of F+ coliphage are 10^5 PFU/g (unpublished data). Using these values, and an aerosolized phage estimation of 1 pfu/m^3 per 1000 pfu/g, to estimate the number of viruses/m^3 of air yields 2 x 10^{-4} viruses/m^3, which is 250 x less concentrated than utilizing the original values yielding 0.05 viruses/m^3 [20]. It was these values that were used to back calculate the rate of aerosolization, using the point/area source models (Fig 5a/b), of viruses/s, and subsequently used to predict the concentration of viruses/m^3 at specific downwind distances under specific wind speeds [20]. Using these new values, downwind

concentrations of viruses/m^3 of air are 250 x less than that of the values originally calculated. For example an originally predicted value using the point source model was 7.5×10^{-3} viruses/m^3 during wind speed of 20 m/s at a downwind distance of 100 m, but through current calculations, this value becomes 3.00×10^{-5} viruses/m^3. This value is then used to establish the number of viruses inhaled/hr exposure, utilizing the equation N = X 0.83 E, where N = the number of viruses inhaled, X = concentration of viruses/m^3, E = time of exposure (hr), and 0.83 is the amount of air inhaled (m^3) by the average person/hr [20]. Thus the number of inhaled viruses corresponding to a 24-hour exposure is 5.98×10^{-4} viruses. The viral risk of infection is described by utilizing the one hit exponential model, $P = 1 - \exp(-rN)$, where P is the probability of infection, r describes the virus ability to infect and overcome host defenses (r=0.0253), and N is the inhaled number of viruses (5.98×10^{-4} viruses) [29]. Thus the risk of infection from a 24-hour exposure to land application of biosolids under a constant 20m/s wind speed would yield a 1.51×10^{-5} risk of infection. Compared to previous calculations, as calculated by Dowd et al, this infectious risk is 5 orders of magnitude less than the reported 1.00 risk of infection. It is important to note that an incorrect (r) value of 39.5 was used, whereas the correct (r) value is 1/39.5, yielding 0.0253 (45). Correctly using this (r) value and using virus downwind concentrations predicted by Dowd et al. yields a risk of 3.76×10^{-3}, which is nearly 3 orders of magnitude less than that of the reported value of 1.00 using the same criteria (100 m downwind, 20 m/s windspeed, and 24 hr exposure).

Conclusions

It is clear from this review that bioaerosols can be generated during wastewater treatment, land applied wastewater, land applied biosolids, and composting sites. Bioaerosols generated by wastewater treatment plants, and composting plants, may not contribute to health effects in the surrounding community, as the majority of the aerosols generated by both plants are maintained to within the site. In addition, some modern wastewater treatment plants and composting plants are currently being built as enclosed structures. This suggests that the majority of aerosols generated at each plant may contribute to the health effects of the workers and handlers only, and to a lesser extent the general public. Hygienic practices need to be employed to reduce the health risks related to work in such an environment. Simple practices, such as the wearing of gloves, washing of hands, and eye protection can minimize direct inoculation of pathogens into the body. Within enclosed wastewater treatment and composting plants, exposure can also be minimized by the usage of air filters in areas of great mechanical agitation. Overall the risk of infection from bioaerosols generated at a wastewater treatment plant, or at a composting plant is low.

Wastewater irrigation or liquid biosolid land application can produce bioaerosols, as these methods result in an aerosol being launched a number of feet into the air particularly with wastewater spray irrigation. Wastewater irrigation is generally considered to be the more likely to result in bioaerosol production, while liquid biosolid land application utilizing a tank truck is considered to be of minimal risk. In addition, the

bioaerosols created by spray of wastewater will more easily be deposited within the lung, and enhanced travel is seen with these droplets when compared to the much larger and denser bioaerosol droplets produced by biosolid spray. Spreading of "cake" biosolids also creates aerosols, but it seems that the loading of these spreaders creates more aerosols than the actual land application. The amount of microorganisms being launched by loading events leads to increased numbers of bioaerosols in the area surrounding the loading site, but overall transport of these microbes over great distances has been shown to be unlikely. Overall, land application of biosolids would appear to create minimal adverse public health affects with respect to bioaerosols. Overall, the risk of infection from bioaerosols generated during land application of biosolids is low.

Once again, as with wastewater treatment and composting plants, the health risk seems to be greater for the workers themselves than for the general public. Therefore common sense hygiene practices should be encouraged in these situations, the use of particulate blocking masks, gloves, and most importantly hand-washing. However exposure also can be minimized through the use of buffer zones, chlorination, storage of wastewater, application during daylight hours with ultraviolet light and dessication acting as methods of disinfection, application devices which minimize aerosol production, usage of higher quality biosolids/wastewater, and application during low wind velocity conditions. To date, few data are available on bioaerosol production during land application of biosolids, and most studies have relied on measurements of bacterial indicators and phage surrogates. Data on enteric pathogens is sparse, particularly with regard to viruses, thus

the need for more research with currently employed techniques such as polymerase chain reaction. There exists multiple research articles on the presence of bioaerosols from wastewater treatment plants, composting plants, and wastewater land application, but still the need for a comprehensive look at the generation of bioaerosols from the land application of biosolids using multiple methods of application needs to be investigated, as this is the area of waste reuse that is garnering the most amount of interest as housing communities are beginning to intrude on land application sites.

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Table 1. Factors Affecting Bioaerosol Fate and Transport

Parameter	Potential to Affect	
	Fate	Transport
Relative humidity	Yes	Yes
Temperature	Yes	No
Wind speed	Unknown	Yes
Ultraviolet radiation	Yes	No
Oxygen concentration	Yes	No
Method of aerosol generation	Yes	Yes

Table 2. Microbial inactivation-constants used in transport modeling of bacteria and viruses.

Aerosolized Microorganism	Inactivation Constant
Rotavirus	2.86×10^{-2} (Ijaz et al., 1985)
Coronavirus	2.66×10^{-2} (Ijaz et al., 1985)
Salmonella sp.	2.35×10^{-4} (Mitscherlich and Marth, 1984)
E. coli	1.92×10^{-4} (Mitscherlich and Marth, 1984)

Source: Adapted from Dowd et al (2000).

Table 3. Aerial Microbial Densities Influenced by Two Types of Aeration Systems used at Wastewater Treatment Plants

	2 m Downwind		10 m Downwind		Upwind
Microorganism	Mechanical Aeration (CFU/m^3)	Diffuse Bubbler (CFU/m^3)	Mechanical Aeration (CFU/m^3)	Diffuse Bubbler (CFU/m^3)	(CFU/m^3)
Total bacterial count	1817	222	1383	105	67
Total fungal count	2900	190	5000	106	92
Staphylococci	100	25	183	11	0
Total coliforms	967	0	367	0	0
E. coli	54	0	17	0	0

All samples collected from the same wastewater treatment plant throughout the summer during different periods of aeration system use: mechanical aeration, diffuse bubbler.

*All values reported in colony forming units/m^3 air
Source: Modified from Brandi et al. (2000).

Table 4. An Example of Downwind Microorganism Densities Caused during Spray Irrigation of Wastewater

Microorganism	All values reported in cfu or pfu/m^3				
		Downwind samples			
	Ambient	30-89 m	90-149 m	150-249 m	250-409 m
Fecal coliforms	<0.006	180.00	1.80	0.70	0.30
Fecal streptococci	0.07	140.00	16.00	8.00	0.50
Mycobacteria	0.1	0.10	0.80	0.20	0.20
Clostrtidium perfringens	0.08	9.00	1.20	1.30	0.60
Coliphage	<0.003	9.90	1.80	0.90	0.10
Enteroviruses		0.05			

cfu = colony forming units
pfu = plaque forming units
Source: Modified from Camann et al. (1988).

Table 5. Types of Applicators Used for Land Application of Biosolids

Method of Application	Biosolid Material	Example Location
Slinger (90 feet) (Figure 1)	Cake (20% solid)	Sunnyside, Washington
Manure spreader (Figure 2)	Cake (20% biosolids)	Solano County, California
Spray tanker (Figure 3)	Liquid (8% solids)	Pima County, Arizona
Spray irrigation (Figure 4)	Liquid (2% solids)	Houston, Texas

Figure 1. Biosolids Slinger Operation

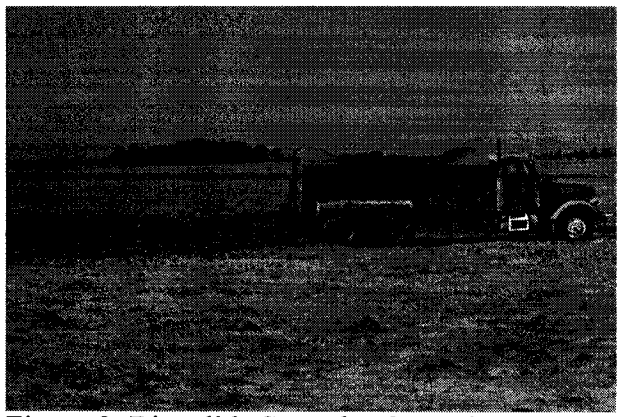

Figure 2. Biosolids Spreader Operation

Figure 3. Biosolids Liquid Spray Tanker Operation

Figure 4. Biosolids Liquid Spray Irrigation

a)

$$X(x,y,z) = \left(\frac{Q\ \varpi}{2\pi\mu (Y\ x/2)(Z\ x/2)} \right) \times \left\{ \exp\left(-0.5 \left(\frac{y}{Y\ x/2} \right)^2 \right) \right\} \times \left\{ \exp\left[-0.5 \left(\frac{z - H}{Y\ x/2} \right)^2 \right] + \exp\left[0.5 \left(\frac{z + H}{Z\ x/2} \right)^2 \right] \right\} \times \exp(-\lambda)$$

Where:

X = concentration of particles/m^3 of air at x, y, and z meters downwind from the source

x = The axis extending along the mean direction of air flow

y = The axis lateral to the direction flow

z = The axis vertical to the direction flow

Y and Z = Plume spread factors or dispersion characterics (m) based upon meterological conditions

H = The source height (m)

Q = The rate of release from the source (particles/s)

ϖ = Constant that accounts for an increase in rate of release from the source with increased wind velocity

μ = The mean wind velocity (m/s)

λ = The factor accounting for microbial inactivation is described by the following equation:

$\lambda = k\ (x/\mu)$

where:

k = The microbial inactivation constant

μ = The distance from the source to the sampling location

Figure 5a Point source modeling equation.
Source: Modified from Dowd et al (2000).

b)

$X\ (x > x_0, y, z) =$

$$\left\langle \frac{Q}{\sqrt{2\pi}\ \mu\sigma_z\{x\}\ y_0} \times \left\{1 + 2\sum_{N=1}^{\infty} \left\{\exp\left[-1/2\left(2nH_m{}^2\right)\right]\right\}\right\} \times \left[\operatorname{erf}\left\{\frac{y_0/2 + y}{\sqrt{2}\sigma_y\{x\}}\right]\right. + \right.$$

$$\left.\left. \operatorname{erf}\left[\frac{y_0/2 - y}{\sqrt{2}\sigma_y\{x\}}\right]\right\}\right\rangle \exp(-\lambda)$$

Where:

x_0 = The along-wind dimension (m) of the source area

y_0 = The cross-wind dimension (m) of the source area

H_m = The estimated depth of the atmospheric mixing layer (m)

σ_{z0} = The vertical source dimension (m)

$\sigma_z\{x\}$ = The molecular diffusion coefficient measured in (m)

$\sigma_y\{x\}$= The molecular diffusion coefficient measured in (m)

$\sigma E'$, $\sigma A'$= Constants based upon lapse meteorlogical conditions

where:

$$\sigma_z\{x\} = \sigma E'\ (x + x_0/2) + \sigma_{zo}$$

$$\sigma_y\{x\} = \sigma A'\ (x + x_0/2) + \sigma_{zo}$$

Figure 5b Area source modeling equation.
Source: Modified from Dowd et al (2000).

APPENDIX B

Estimation of Bioaerosol Risk of Infection to Residents Adjacent to a Land Applied Biosolids Site using an Empirically Derived Transport Model[#]

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Running Headline: Bioaerosols and Biosolids

[#] Accepted for publication by the Journal of Applied Microbiology (2004)

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SUMMARY

AIM: The purpose of this study was to develop an empirically derived transport model, which could be used to predict downwind concentrations of viruses and bacteria during land application of liquid biosolids and subsequently assess microbial risk associated with this practice.

METHODS AND RESULTS: To develop the model, coliphage MS2 and *Escherichia coli* were aerosolized after addition to water within a biosolids spray application truck, and bioaerosols were collected at discrete downwind distances ranging from 2 m to 70 m. Although coliphage were routinely detected, *E. coli* did not frequently survive aerosolization. Data on aerosolized coliphage was then used to generate a virus transport model. Risks of infection were calculated for various ranges of human virus concentrations that could be found in biosolids.

CONCLUSIONS: A conservative estimate at 30.5 m (assumed to be nearest adjacent residences) downwind, resulted in risks of infection of 1:100,000, to the more realistic 1:10,000,000 per exposure. Conservative annual risks were calculated to be no more than 7:100,000 where as a more realistic risk was no greater than 7:10,000,000. Overall, the viral risk to residences adjacent to land application sites appears to be low, both for one time and annual probabilities of infection.

SIGNIFICANCE AND IMPACT OF STUDY: This study demonstrated a simple approach towards modeling viral pathogens aerosolized from land applied liquid biosolids, and offers insight into the associated viral risk.

Keywords: biosolids, sludge, pathogen, risk, aerosol, coliphage

INTRODUCTION

In the United States, greater than 60% of all biosolids produced are land applied (National Research Council 2002). Though this process has occurred for decades, it has recently come under intense scrutiny from communities near land application sites. Biosolids are the treated solid by-product of wastewater treatment, which routinely contain pathogenic microorganisms. Biological aerosols (bioaerosols) are biological particles, including pathogenic microorganisms, which have become aerosolized through either human activity such as the land application of biosolids, or through natural activities such as the dispersion of fungal spores. The generation of biological aerosols from the land application of biosolids has not been well studied. Pathogenic microorganisms such as *Salmonella*, norovirus, and hepatitis A virus can all potentially be aerosolized from biosolids during land application. Despite questions regarding bioaerosols generated from the land application of biosolids, most have remained unanswered, due to a lack of field-generated data.

A study published by Sorber et al. 1984 stated that there was little to no risk from the land application of biosolids with respect to bioaerosols. In this study, the liquid biosolids were land applied via a high-pressure liquid spray gun and a spray truck, and bioaerosols from both types of land application were then compared. It was found that the majority of the aerosols were generated by the spray gun, with less aerosolization from the spray trucks. The height of the spray truck's exit port was given as a possible reason for the minimal detection of bioaerosols from the spray truck. Despite the

presence of enteroviruses in the biosolids, none were detected in any aerosol sample via cell culture.

A more recent study conducted by Pillai et al. 1996, focused on the aerosolization of indicator microbes such as total coliforms, fecal enterococci, and male specific coliphage from a "cake" biosolids land application site. The investigators were only able to detect indicator microbes on a few occasions and concluded that little risk was associated with bioaerosols generated during the land application of biosolids.

This raised questions as to the efficacy of the aerosol sampling protocol to detect low levels of aerosolized biological agents, ie. were there no bioaerosols, or did the method of detection lack sensitivity? To answer this question, some type of positive control is needed such as can be produced by the aerosolization of known concentrations of microbes from a liquid. The study of seeded microorganisms aerosolized from water is not an uncommon practice, as many studies have monitored *Escherichia coli* aerosolized from secondary treated wastewater irrigators (Teltsch and Katzenelson 1977; Teltsch et al. 1980a; Teltsch et al. 1980b). The study (Teltsch et al. 1980b) found that aerosolized *E. coli* was reduced by 90% within the first two min of aerosolization during favorable conditions (low temperatures, high relative humidity), compared to unfavorable conditions (high temperatures, low relative humidity), where aerosolized *E. coli* concentrations were reduced by 90% within the first 10 seconds of aerosolization.

The purpose of this current study was to aerosolize seeded *E. coli* and coliphage (MS-2) from pumped non-chlorinated groundwater (seeded water), and to monitor bioaerosols generated from a system similar to a liquid biosolids spray applicator. Inoculation

(seeded) concentrations were chosen that would mimic biosolid concentrations of coliphage and *E. coli*. Recent studies have shown that concentrations of coliphage are greater per dry gram of biosolids than previously believed. In a recent study by Chetochine et al. (2004), it was shown that following 40+ sequential extractions, coliphage were continuously removed from biosolids. Therefore, it was hypothesized by the authors of the study that viruses are embedded within or absorbed to the surface of biosolid solid particles. Because of this, we believe that only those viruses in liquid phase are available to be aerosolized. For seeded water experiments, seed concentrations included levels of coliphage found in biosolids, and in addition, by increasing seed concentrations, potentially greater aerosol production could occur, allowing for enhanced detection and greater modeling precision. Using seeded water to produce aerosolization allowed for the generation of fate and transport data with respect to bacteria and coliphage. This data was subsequently used to derive an empirical transport model to estimate bacterial and viral transport as bioaerosols, following land application of liquid biosolids.

MATERIALS AND METHODS

Stock microorganisms

E. coli ATCC 15597 (American Type Culture Collection; Manassas, VA) was used to culture coliphage MS-2. Stock concentrations of MS-2 were cultured to a concentration adequate to seed 8,000 L of groundwater at a final concentration of 1×10^6 PFU mL^{-1}. An aliquot of stock MS-2 (0.1 mL) was first placed into individual disposable culture tubes each containing four mL of top agar (30g TSB, 10 g Bacto agar per L) (Becton

Dickinson; Sparks, MD). Approximately 100 culture tubes were subsequently plated onto petri dishes containing TSA (tryptic soy agar) (Becton Dickinson; Sparks, MD) and incubated for 16 hours at 35° C. Following incubation, each plate was washed with 10 mL Tris buffered saline (pH, 7.2) at room temperature with continuous swirling every 15 min. The wash was then removed via pipet, at which time it was purified via vacuum filtration (0.20 μm) utilizing Nalgene filtration units (Nalge Nunc Int; Rochester, NY), typically yielding a concentrated solution (10-12 $\log_{10}$ PFU mL^{-1}).

E. coli ATCC 25922 (American Type Culture Collection; Manassas, VA) was used as a representative bacterium, and was cultured to a concentration adequate enough to seed 8,000 L of ground water at a final concentration of 1×10^5 cfu mL^{-1}. Initially a single colony lifted from a stock Petri dish was used to begin an overnight culture containing 100 mL TSB (tryptic soy broth) (Becton Dickinson; Sparks, MD). This culture was then used to seed approximately 20 L of sterile TSB contained within a 20 L plastic bucket (Plastican, Leominster, MA). Each bucket was subsequently placed into a walk-in incubator for 24 h at 35° C. Approximately 80 L (8 $\log_{10}$ CFU mL^{-1}) of *E. coli* ATCC 25922 was produced using this method.

Spray application method

A Better Built (Fig 1) spray tanker (Better Built Equipment; Alpharetta, GA) with a maximum liquid capacity of 17,000 L was used to aerosolize the seeded water. The seeded water plume was aerosolized at a relative height of four m above the ground, with a width of four m, and a length of approximately three m behind the spray tanker. The

tanker traveled at a rate of five km h^{-1}, and was used to land apply seeded water, while samples of air were collected and subsequently analyzed for biologicals.

Seed water preparation

The application tanker was loaded with non-chlorinated groundwater used for irrigation and the microorganisms were subsequently added to the water in the tanker. This mixture was then homogenized by continuous driving of the tanker truck for approximately five min. Two seed water samples were collected prior to initiation of aerosolization and post aerosolization, which were subsequently analyzed for coliphage and *E. coli*.

Bioaerosol samples

Bioaerosol samples were collected via the use of six SKC Biosamplers® (SKC-West Inc.; Fullerton, CA) operating at an airflow rate of 12.5 L min^{-1} provided through the use of Vac-U-Go sample pumps (SKC-West Inc.; Fullerton, CA). All Biosamplers® were sterilized via the use of a steam autoclave prior to field sampling. The sterile samplers were placed onto surveying tripods (Seco Mfg.; Redding, CA) raised to a height so that the intake nozzle simulated the average human breathing height of 1.5 m (American Society for Testing Materials 1993). Samplers were located perpendicular to the wind vector and parallel to the travel vector of the spray tanker. A total of six samplers were used for sample collection, and were arranged as three sets of duplicate samples located at discrete sample distance points, comprising a single sample series array (Fig 2). Prior to operation, the samplers were loaded with 23 mL of microbial trapping fluid consisting of 0.1 % peptone (Becton Dickinson; Sparks, MD) water amended with antifoam agent B

(Sigma-Aldrich; St. Louis, MO). These were operated for a total of 20 min beginning one min prior to exposure of the aerosol plume, following the spraying of the seeded water. Samples were aseptically removed via the use of sterile polystyrene pipets (Corning; Acton, MA) and were transferred to sterile polypropylene 50 mL centrifuge tubes (VWR; West Chester, PA). All samples were placed on ice during transport to the laboratory. Between sample runs, Biosamplers® were disinfected utilizing a 70% ethanol solution. Samples were then transported back to the laboratory and analyzed within six h. Prior to analysis, all sample volumes were measured and standardized to the original 23 mL start volume to account for evaporation. All samples were vortexed for one min following standardization. Samples were collected on five separate d. Environmental conditions were monitored via the use of a Kestrel Pocket Weather Meter (Nielsen-Kellerman; Boothwyn, PA) during collection of the aerosol samples.

Microbial assays

Coliphage was assayed via the use of the double agar overlay method (Adams et al. 1959). A total of five mL of the sample buffer was screened for the presence of coliphage capable of infecting *E. coli* ATCC 15597 (American Type Culture Collection; Manassas, VA). Sample plates were incubated at 35° C for 24 h. Coliphage aerosol concentrations were determined as Plaque Forming Unit (PFU) m^{-3} air.

E. coli was assayed using Colilert substrate technology (IDEXX; Westbrook, ME) coupled with Colitray® for Most Probable Number (MPN) enumeration system (American Public Health Association et al. 1998). A total of 10 mL of the sample buffer was assayed. Samples were incubated at 35° C for 24 h at which time yellow color

formation and fluorescence was noted for each sample tray. MPN was determined as MPN m^{-3} air.

Statistics

Statistical analysis was performed through the use of Microsoft Office 2000, Excel spreadsheet analysis tools.

RESULTS

Development of a linear regression model

Table 1 shows the factors that were statistically significant with respect to bioaerosols containing coliphage via analysis of variance. Initial microbial concentrations within the tanker, temperature, and windspeed when compared to aerosolized coliphage concentrations demonstrated an influence on the aerosolized coliphage. Of these three factors, the $\log_{10}$ of seed concentrations and windspeed in m s^{-1} were used to normalize the phage data, which was subsequently used to generate an empirical transport model (Fig 3). When aerosolized coliphage were not detected, the detection limit (9.2 PFU m^{-3}) was used in the linear regression model.

In the case of coliphage, aerosolized levels greater than detection limits were detected at downwind distances up to and including 60 m, whereas aerosolized *E. coli* concentrations were lower, and found only at distances close to the application site (Table 2). Only upon one sampling occasion was *E. coli* detected at distances greater than two m from the point source. Most often, *E. coli* was below the detection limit of 4.6 MPN m^{-3}. Because of this, aerosolized *E. coli* data was not used to generate a linear regression, since insufficient data points were available.

To generate the coliphage regression model, a minimum of four samples were used at each individual sample distance, for a total of 70 aerosol samples at 10 different sample distance points from two m to 70 m. Samples were analyzed and values were pooled together, following analysis, to generate one number. The individual sample points as seen in the linear regression is an average of all sample points collected at that specific downwind distance. The best-fit curve is shown in Figure 3, ($r^2 = 0.67$, r=0.82). Field measurements indicated that as windspeed increased, downwind concentrations of coliphage increased. For this reason windspeed, of the environmental factors, was thought too most significantly affect fate and transport of phage.

Risk assessment modeling – predicted aerosolized coliphage concentrations

The linear regression model was used as the basis of the microbial risk analysis, as it was believed to be predictive of microbial aerosol concentrations at specific downwind distances. From the model depicted in Figure 3, predicted values of coliphage were derived at downwind distances of between two and 1,000 m downwind from a hypothetical biosolids application site. Coliphage values were derived by inputting "x" values, in meters downwind from the point source, into the linear equation, $y = -0.0022 x + 0.1849$. The yielded value was then adjusted to account for normalization for windspeed and seed concentration, hence the y values must be multiplied by the average windspeed and $\log_{10}$ (seed concentrations) used to generate the model, in this case 2.29 m s^{-1} and 7.24 respectively, yielding a $\log_{10}$ coliphage concentration per m^3 air at a specified distance.

Risk assessment modeling – estimated aerosolized virus values

Coliphage values, once determined, were used to estimate concentrations of aerosolized pathogenic viruses, specifically coxsackievirus. Coxsackievirus A21 was chosen to estimate viral risk, as it is the only enteric virus for which inhalation dose response data exists (Couch et al. 1965). We assumed that any human virus selected would aerosolize and be transported with the same efficiency as the modeled coliphage virus, despite being aerosolized from biosolids rather than water. Studies on the aerosolization of bacteria and viruses from liquid biosolids (data not shown) and from "cake" biosolids resulted in minimum detection of aerosolized microbes (Pillai et al. 1996). Thus our modeling approach overestimates the risk and provides a conservative approach towards the risk analysis. Secondly a ratio of virus to coliphage present in biosolids was calculated to estimate the number of human pathogenic viruses aerosolized. Ratios used varied from conservative, i. e. a ratio of one to 10,000 representing one animal virus for every 10,000 coliphage per gram, to the more realistic ratios of virus to coliphage values based on reported literature (Gerba et al. 2002). Past studies have reported concentrations of pathogenic viruses ranging from one MPN g^{-1} to 300 MPN g^{-1}, but due to more efficient treatment during Class B biosolids production, the concentration is most likely to be equal to or below one MPN/g (Gerba et al. 2002). A coliphage concentration of 1×10^5 PFU g^{-1} (dry) of biosolids was chosen as the default concentration of coliphage in biosolids based on recent studies (Chetochine et al. 2004). Based on the method of aerosolization of the seed water, this modeling approach lends itself best for liquid biosolids that are sprayed in the field. Given the percent solids in a biosolids sample

(assuming eight percent), it was determined that the amount of coliphage per mL of biosolids was 8×10^3 PFU mL^{-1}, which is the equivalent of a coliphage concentration of 1×10^5 PFU g^{-1} (dry). Since the concentration of coliphage in the seed water was known, a ratio could be drawn between aerosol values generated by the linear regression model and estimated values of aerosolized coliphage generated by a hypothetical biosolids land application operation. To generate this value, a ratio was first drawn between coliphage concentrations in the source material (seed water) to values predicted by the empirical model. Subsequently the same ratio was applied to a hypothetical land applied site using biosolids as the source material and an unknown "x" value, the aerosolized coliphage from this hypothetical site. Once these two ratios were known, a proportion between the two was applied to solve for "x". Once coliphage values were known, aerosolized human virus concentrations could be calculated utilizing the ratio between concentrations of virus to coliphage, i.e. 1:10,000 present in the biosolids, assuming all viruses will aerosolize with the same efficiency (Table 3).

Risk assessment – modeling

Risk of infection modeling was performed using the one-hit exponential model (Haas et al. 1999), $p_i = 1 - \exp(-rN)$, where:

'r' = a constant describing the organisms' ability to infect and overcome the host ($r = 0.0253$, $r = 1/39.4$) (Couch et al. 1965)

'N' = the exposure dose in number of organisms

This model was chosen, as it most accurately describes the dose response to a one time coxsackievirus aerosol exposure.

The exposure dose is described as, $N = x * 0.83 * t$, where:

'x' = the number of organisms per m^3

0.83 m^3 h^{-1} = the average human breathing rate (Environmental Protection Agency, 1997)

't' = the exposure duration in h

The annual risk model is described as, $p_{(annual)} = 1 - (1 - p_i)^d$, where

'p_i' = the one time probability of infection, described above

'd' = the number of days exposed per year

Occupational risk

Occupational risk is described here as risk of infection posed during exposure to pathogenic microbes during an occupational setting (i.e. biosolids workers). To utilize a conservative exposure, it was assumed that an individual would remain downwind of a biosolids application site for either one or eight h. Risk of infection, for coxsackievirus, was calculated for both exposure scenarios at multiple downwind distances (Table 4). Exposure was assumed to be constant throughout the entire exposure period, although recent studies have shown that exposure to bioaerosols from spray land application occurs as a pulse of short duration, less than one minute per pass (Tanner et al. 2004).

Assuming 0.1 virus g^{-1} of biosolids, an eight h exposure, and two m downwind yielded a risk of 1.31×10^{-6}, more accurately estimating the one time risk posed to biosolids handlers. A more conservative modeling approach, which assumes 10 infectious viruses g^{-1} of biosolids, eight h exposure, and two m downwind of the point source, yielded an infection risk of 1.31×10^{-4}.

Community risk

To assess a community infection risk, it was assumed that anyone living at least 30.5 m (100 ft) from a biosolids land application site would be exposed to biological aerosols originating from the point source. Assuming 0.1 virus g^{-1} and an eight h exposure, residing at least 30.5 m downwind from the point source, yielded a community risk of infection of 1.20×10^{-7}. This risk of infection is hypothesized to most accurately reflect the risk experienced by an individual residing downwind from an application site. A more conservative approach, which assumes an eight h exposure, 30.5 m downwind, and 10 infectious viruses g^{-1}, yielded a one time risk of infection from aerosolized coxsackievirus to be 1.20×10^{-5}. As would be expected, infection risks decreased with increased downwind distances from the modeled point source (Fig 4).

The above risk values only represent one-time risks of infection. The annual risk posed towards individuals living near a liquid biosolids land application site can be estimated based on annual application rates. Land application of biosolids typically only takes place once or at most twice a year in a specific location. To estimate an annual risk posed to an individual, we assumed an annual exposure based on 6 days, 2 applications and 3 days per application. The annual infection risks are shown in Table 5. A realistic approach predicts a risk of 7.22×10^{-7} infections per year assuming an 8 h exposure, 30.5 m or 100 ft downwind, and 0.1 virus g^{-1} biosolids. An annual risk of infection based on conservative assumptions (8 h exposure, 10 viruses g^{-1}) at a distance of 30.5 m, yields a risk of 7 infections per 100,000 exposed people per year.

DISCUSSION

This study suggests that bacteria particularly Gram negative are inactivated much more quickly than viruses and this leads to a lower risk of infection. This phenomenon has been previously demonstrated (Teltsch et al. 1980b; Heidelberg et al. 1997). Overall community risk associated with aerosolized bacteria, specifically aerosolized Gram negative bacteria such as *E. coli*, is extremely low as *E. coli* aerosol concentrations were below detectable levels at distances greater than 20 m from the application site. Hence, risk values associated with aerosolized Gram negative bacteria such as *E. coli* would tend to be less than the stated viral risk values according to this approach. In developing the model, it was found that of all the measured environmental factors, windspeed most affected the transport of aerosolized coliphage. The effect of windspeed on aerosol concentrations has been documented from wastewater treatment plant trickling filter towers (Goff et al. 1973) and wastewater aerosolization (Smith et al. 1999). Temperature also affected aerosol concentrations, most likely due to its influence on microbial inactivation (Israeli et al. 1994). The influence of relative humidity has been documented regarding biological aerosols (Israeli et al. 1994), but during the development of the linear model relative humidity did not significantly have a role in bioaerosol concentrations, most likely due to lack of variation in measured relative humidity.

In our recent field studies, it was determined that overall aerosol microbial concentrations during the land application of liquid biosolids were consistently below detection levels (data not shown), whereas during the land application of seeded water, aerosol concentrations of coliphage were consistently detected at downwind distances up

to 60 m. Therefore it appears that the physical and chemical properties of biosolids, specifically the presence of viral binding proteins and human tissue present in biosolids, can inhibit or reduce viral aerosolization (Sano, D. 2003).

Overall, one time and annual risks of infection from aerosolized virus appear to be insignificant at distances greater than 30.5 m or 100 feet downwind of a biosolid application site using realistic concentrations of human pathogenic viruses present in biosolids. Using an estimated viral concentration of 0.1 virus g^{-1} of biosolids resulted in an estimated risk less than one infection per million exposed d^{-1}. Infection risks using conservative virus concentrations present in biosolids would tend to overestimate the chance of infection, but are necessary to ensure adequate safety. Even over estimation of viral concentrations in biosolids by two orders of magnitude yielded risks of infection equivalent to one per hundred thousand individuals exposed d^{-1}.

Setback distances of 100 feet appear to be adequate based on this study, as viruses traveling beyond 30.5 m or 100 feet appear to be inactivated quickly based on this empirically derived model. Risks at these distances and beyond are predicted by this study to be below one in 10,000. Annual risks of infection assuming two application periods per year yielded risks well below one in 10,000, the acceptable annual risk proposed by the Environmental Protection Agency for drinking water (Regli et al. 1991). Populations at these distances (> 30.5 m or 100 ft) are at a minimal risk of infection based on these estimates. In summary, based on these risk analyses, the likelihood of an individual in an adjacent community becoming infected as a result of a bioaerosol during land application of liquid biosolids is minimal.

ACKNOWLEDGEMENTS

This work was funded by the National Science Foundation Water Quality Center located at the University of Arizona.

Table 1. Factors affecting coliphage aerosol concentrations based on analysis of variance.

Factor	Association with Aerosolized Coliphage: (P value < 0.05)	
Temperature	Yes	P = 0.03
Wind Speed	Yes	P = 0.003
Relative Humidity	No	P = 0.12
Seed Concentration	Yes	P = 0.0002

Table 2. Aerosolized *E. coli* concentrations and coliphage (MS2) concentrations following aerosolization from a seeded water tanker.

	Detected Aerosol Concentration Range‡	
Downwind distance from application site (m)	*E. coli* MPN* m^{-3}	MS2 PFU m^{-3}
2	BDL† – 4.6	27.6 – 267
5	BDL– 4.6	BDL – 405
10	BDL – 1,370	36.8 – 221
15	BDL	55.2 – 276
20	BDL	27.6 – 331
30	BDL	BDL
40	BDL	DL – 368
50	BDL	BDL
60	BDL	BDL – 258
70	BDL	BDL

*MPN – Most probable number, PFU – Plaque forming unit per m^3 air sampled

†BDL – Below Detection Limit, DL – Detection Limit

‡All values are actual reported results, (not normalized to wind speed or seed concentrations)

Table 3. Virus to coliphage ratios used to generate associated aerosolized human virus from a hypothetical land application site.

Distance (m)	Predicted human virus concentrations (virus m^{-3})		
	Virus : Coliphage Ratio		
	$1:10^6$ 0.1 virus g^{-1}*	$1:10^5$ 1 virus g^{-1}	$1:10^4$ 10 viruses g^{-1}
2	7.83×10^{-06}	7.83×10^{-05}	7.83×10^{-04}
10	4.00×10^{-06}	4.00×10^{-05}	4.00×10^{-04}
30.5	7.16×10^{-07}	7.16×10^{-06}	7.16×10^{-05}
100	2.10×10^{-09}	2.10×10^{-08}	2.10×10^{-07}
500	5.52×10^{-24}	5.52×10^{-23}	5.52×10^{-22}
1000	3.29×10^{-42}	3.29×10^{-41}	3.29×10^{-40}

* Concentration based on human virus per dry g of biosolids.

Table 4. Risk of infection from coxsackievirus A21 hypothetically aerosolized from land applied biosolids based on estimated number of human enteric viruses present in Class B biosolids.

Distance (m)	Risk of Infection†			
	0.1 virus g^{-1}*	1 virus g^{-1}	10 viruses g^{-1}	
2	**1.64×10^{-07}**	**1.64×10^{-06}**	**1.64×10^{-05}**	Exposure times: 1h/ 8h‡
	1.31×10^{-06}	**1.31×10^{-05}**	**1.31×10^{-04}**	
10	8.40×10^{-08}	8.40×10^{-07}	8.40×10^{-06}	
	6.72×10^{-07}	6.72×10^{-06}	6.72×10^{-05}	
30.5	1.50×10^{-08}	1.50×10^{-07}	1.50×10^{-06}	
	1.20×10^{-07}	1.20×10^{-06}	1.20×10^{-05}	
100	4.40×10^{-11}	4.40×10^{-10}	4.40×10^{-09}	
	3.52×10^{-10}	3.52×10^{-09}	3.52×10^{-08}	
500	0§	0	0	
	0	0	0	
1000	0	0	0	
	0	0	0	

*Concentration based on virus per dry g of biosolids.
†Bold values represent risks greater than 1:10,000
‡Top value: risk from 1 h exposure; Bottom value: risk from 8 h exposure
§Zero values are equivalent to risk of infection, $< 4.40 \times 10^{-11}$

Table 5. Community (those living > 30.5 m) annual risk of viral infection from coxsackievirus A21 hypothetically aerosolized from land applied biosolids based on two 3-day applications per year.

Distance (m)	Risk of Infection			
	0.1 virus g^{-1}*	1 virus g^{-1}	10 viruses g^{-1}	
30.5	9.02×10^{-08}	9.02×10^{-07}	9.02×10^{-06}	Exposure times: 1h/8h†
	7.22×10^{-07}	7.22×10^{-06}	7.21×10^{-05}	
100	2.64×10^{-10}	2.64×10^{-09}	2.64×10^{-08}	
	2.11×10^{-09}	2.11×10^{-08}	2.11×10^{-07}	
500	0‡	0	0	
	0	0	0	
1000	0	0	0	
	0	0	0	

*Concentration based on virus per dry g of biosolids.
†Top value: risk from 1 h exposure; Bottom value: risk from 8 h exposure
‡Zero values are equivalent to risk of infection, $< 2.64 \times 10^{-10}$

Figure 1. Land application of seeded water using a spray tanker.

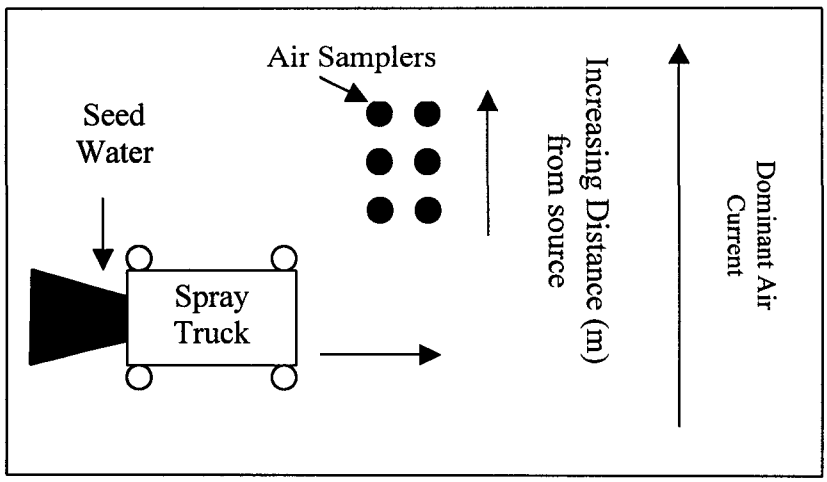

Figure 2. Bioaerosol Sampling Strategy (not to scale)

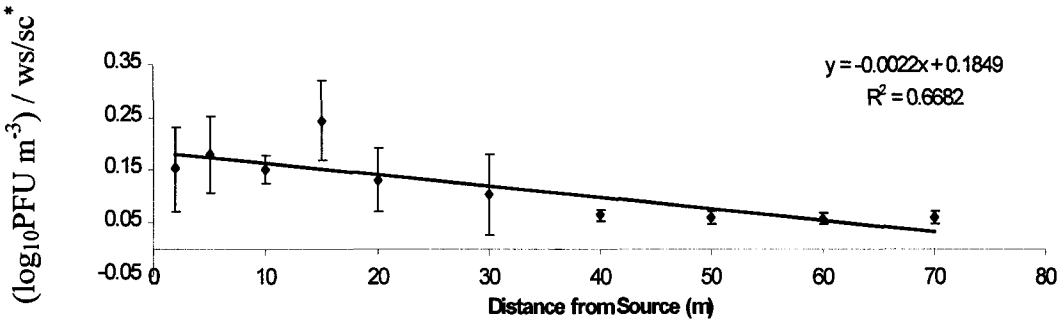

Figure 3. Aerosolized coliphage transport with respect to distance from point source, normalized for seed concentration and wind speed.

* ws = windspeed (m s^{-1}); sc = seed concentrations (log_{10} PFU mL^{-1})

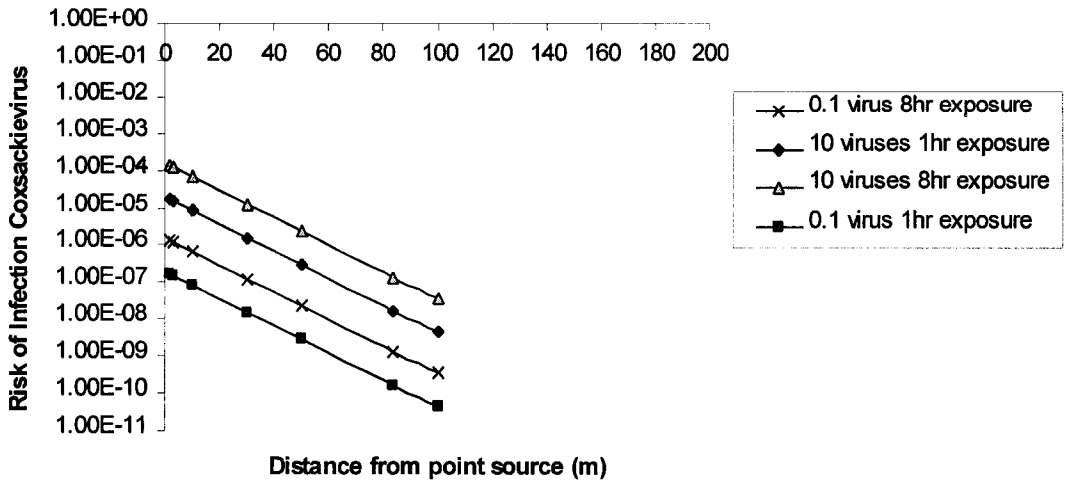

Figure 4. Risk expressed with respect to distance from a hypothetical point source, land applied liquid biosolids.

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APPENDIX C

A National Study on the Incidence of Biological Aerosols from the Land Application of Biosolids: Microbial Risk Assessment[#]

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To be submitted to the Journal of Applied Microbiology (2004)

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SUMMARY

AIMS: The purpose of this study was to evaluate the risk of infection from bioaerosols generated during land application of biosolids. Aerosol samples were collected throughout the continental USA from different biosolids land application sites.

METHODS AND RESULTS: Approximately 350 aerosol samples from 10 sites located throughout the USA were collected via the use of 6 SKC Biosamplers®. Downwind aerosol samples from biosolids loading, unloading, land application, and background operations were collected from all sites. All samples were tested for HPC bacteria, total coliform, *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, although biosolids loading operations resulted in the largest concentrations of aerosolized microbial indicators. Microbial risk analyses was conducted on loading and application scenarios and their subsequent community exposures.

CONCLUSIONS: Overall maximum risks occured during loading operations, although these risks were minimal, not exceeding an annual risk of infection of $4{:}10^{4}$. Land application of biosolids resulted in risks that were less than $9{:}10^{9}$. Overall bioaerosol exposure from biosolids operations poses little community risk based on this study.

SIGNIFICANCE OF STUDY: This study demonstrated the overall incidence of aerosolized microorganisms from the land application of biosolids and subsequently microbial risks of infection were low.

Keywords: Biosolids, risk, pathogens, aerosol, bioaerosol

INTRODUCTION

Concerns about the link between biological aerosols associated with the land application of biosolids and the incidence of illness amongst neighboring communities has received recent public attention (Fackelmann, K 2002). While no evidence exists establishing this link, several communities near land application sites have lodged complaints against their respective counties (National Research Council 2002). The Environmental Protection Agency (EPA) established regulations regarding the treatment, disposal, and reuse of biosolids as a fertilizer (National Research Council 2002).

A limited number of studies have been conducted on the generation of bioaerosols from biosolids land application. Notably, Sorber et al. (1984) stated that little to no risk was associated with the land application of liquid biosolids based on the lack of pathogenic viral presence in a large volume of sampled air. Pillai et al. (1996) and Dowd et al. (2000) focused on the 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 non-traditional 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, F. 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 United States. 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 ten sites across the continental United States 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 windspeeds. Samples were collected from Feburary, 2002 through 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 biosolids involved in this study were at least 15% dry mass "cake" biosolids, although sites such as Houston, TX applied liquid 2% dry mass biosolids. Biosolids application involves

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". 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 parallel to the wind vector and direction of loading. Samples collected during application events are designated as slinging, or spreading samples. This entailed the physical land application of the biosolids. Since this approach involved a moving point source, samplers were aligned parallel to the travel vector and perpendicular to the wind speed vector. Typically biosolids were unloaded on site directly onto the soil or vegetation. Samples collected during unloading stages involved the unloading of the biosolids on-site typically from a "dump truck".

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. Table 1 lists the number of samples collected at each site, in addition to the placement of samples with relation to the aerosol sources. Overall samplers were placed 2 per distance at 3 separate distances per operation comprising 6 simultaneously collected samples. Alternatively samplers were placed 3 per distance at 2 separate distances per operation. Samples collected at 2 m were directly downwind of loading/unloading events, whereas during application operations, a 2 m sample refers to downwind of the biosolids application perimeter.

Site 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) 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). Biosolids were launched from the applicator approximately 30 m into the air. This approach provided two different opportunities for sample collection, specifically samples were collected from "loading" and "slinging" sites.

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 approximately one meter above the ground and ten meters 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 farm via the use of a Fecon Aerospreader® (Fecon Inc; Cincinnati OH), 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

Hopps fields were applied with "cake" biosolids via the use of a biosolids Knight Protwin® slinger (Kuhn Knight Inc; Brodhead, WI). 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; Brodhead, WI). Samples were only 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 approximately 10 m. Samples were collected during the spray application events.

Site 10: Chicago, IL

Cake biosolids were land applied via the use of a modified AgChem Terragator® manure spreader (AgCo; Jackson MN). Samples were collected following post 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). Vac-U-Go ® sampling pumps (SKC-West Inc.; Fullerton, CA) 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) (ASTM 1993). Samples were collected for a total of 20 minutes, or approximately 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). 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 minute. Weather conditions were monitored through the use of a Kestrel portable weather monitor (Nielsen-Kellerman; Boothwyn, PA).

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) were dried in a convection oven at 104° C for 24 hr to ascertain solid percentage and hence dry mass. All data was reported as per dry g. Prior to analyses, biosolids samples were homogenized by placing 10 moist g into 95 mL 0.1% peptone water. This

peptone water extract mixture was shaken via a Labline multiwrist shaker (Barnstead Int; Dubuque, IA) for 30 min on medium setting, and serially diluted to accommodate HPC, *C. perfringens*, Total Coliform, and *E. 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 Standard D 4994-89 Vol 11.02 1993 (554-558) for the extraction of human enteric viruses from dry 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) and incubated at 25° C for 7 days. 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 stated 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 (American Type Culture Collection; Manassas, VA) was assayed utilizing the double agar overlay technique (Adams, M.H. 1959). A total of four mL from the aerosol sample was assayed utilizing

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 hours for aerosol samples to 16 hours 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 *Escherichia coli*

Aerosolized total coliform and *Escherichia 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 et al. 1998). A total of five 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). 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) (Arnon, R and Payment, P 1988). A total of five mL of the aerosol sample was filtered through a membrane filter (0.45 μm) and asceptically transferred to the media. Petri dishes were then incubated for 1-2 days at 44.5° C in an anaerobically sealed jar (Becton Dickinso Microbiology Systems; Sparks,

MD), anaerobic conditions were provided by GasPak Plus (Becton Dickinson Microbiology Systems; Sparks, MD). 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 eight mL portion of the aerosol sample was frozen at – 20° C. Prior to RNA extraction, this aliquot was first concentrated using commercially available Centriprep 50 concentrators (Millipore; Billerica, MA) operating at a speed of 1500 x g for 5 minutes followed by a second spin of 1000 x g for 5 minutes. This yielded a final volume of between 0.6 mL and one mL. In addition to these samples, select aerosol samples were concentrated in their entirety (23 mL) to a final concentrate of between 0.6 mL and one mL. All samples were then RNA extracted using commercially available Qiagen viral RNA extraction kits (Qiagen; Valencia, Ca) as described by the manufacturer. An aliquot of 280 µL of concentrated sample was extracted using these kits and concentrated to a final volume of 80 µL.

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).

Enteroviruses/Hepatitis A Virus RTPCR protocol:

RTPCR was performed through the use of Qiagen One Step RTPCR kits (Qiagen; Valencia, Ca) under the following conditions: RNA was transcribed via a single pre-PCR step of 30 minutes at 50° C, followed by a single step of 15 minutes at 95° C. A three step PCR process, 35 cycles total, began with a cDNA denature step performed at 94° C for 45 seconds; primer annealing was performed at 53° C for 30 seconds, followed by DNA extension at 72° C for 1 minute. 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 (Sigma Genosys; The Woodlands, TX), with previously described sequences (Schwab et al. 1996) to amplify a 197 bp product and 192 bp product for enteroviruses and hepatitis A virus respectively. A final primer concentration of 0.6 μM was achieved. A final volume of 40 μL with 10 μL of template constituted the final tube volume of 50 μL.

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. Hepatits A Virus amplicons were amplified via the use of a second reamplification, employing both primers from the original PCR. A 10 μL 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 Biosytems; Foster City, CA) activation step of 10 minutes at 95° C, followed by 30 cycles of amplification, denature step of 30 seconds at 95° C, and a combined primer

annealing/extension step of 72° C for 45 seconds followed by a final extension step of 72° C for 10 minutes. Reagents were added in the following concentrations and volumes for enterovirus secondary amplification: sterile PCR water (28.45 μL), 10X PCR buffer II (Applied Biosytems; Foster City, CA) (5 μL), 25 mM $MgCl_2$ (Applied Biosytems; Foster City, CA) (5 μL), 10 mM DNTP solution (1 μL), 5 U/μL Amplitaq Gold® (Applied Biosytems; Foster City, CA) (0.25 μL), and each primer 200 μM (0.15μL) to constitute a final volume of 50 μL. Reagents were added in the following concentrations and volumes for HAV secondary amplification: sterile PCR water (32.00 μL), 10X PCR buffer II (4.5 μL), 25 mM $MgCl_2$ (2.4 μL), 10 mM DNTP solution (0.5 μL), 5 U/μL Amplitaq Gold (0.30 μL), and each primer 200 μM (0.15μL) to constitute a final volume of 50 μL. All samples were analyzed in duplicate.

Norovirus RTPCR protocol

Qiagen One Step RTPCR kits were utilized 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 μL 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 minutes at 95° C, followed by 30 cycles of amplification, denature step of 30 seconds at 95° C, primer annealing of 30 seconds at 50° C, and an extension step of 72° C for 30 seconds followed by a final extension step of 72° C for 10 minutes. Reagents were added in the following concentrations and volumes: sterile PCR water (30.5 μL), 10X PCR buffer II (4.5 μL), 25 mM $MgCl_2$ (2.4 μL), 10 mM DNTP solution (0.5 μL), 5

U/μL Amplitaq Gold (0.5 μL), and 50 μM primer MJV12 (1.0 μL), 50 μM primer RegA (0.6 μL) to constitute a final volume of 50 μL. All samples were analyzed in duplicate.

Visualization:

An aliquot of 10 μL of the final double round PCR product was loaded into a 3.5 % 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; Valencia, Ca). Sequences were analyzed 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.

RESULTS

Biosolids

Collection of Class B biosolids from multiple sites throughout the country showed that samples were similar in microbial quantity and quality (Table 2). In general, with the exception of two samples (Houston, TX, Leesburg, VA) most biosolids samples contained the following approximate concentrations of HPC bacteria (10^9 g^{-1}), total coliforms (10^5 g^{-1}), *E. coli* (10^4 g^{-1}), *C. perfringens* (10^6 g^{-1}), and coliphage (10^4 g^{-1}).

Aerosol samples

Cultural analyses from all sites is presented in Table 3 and 4. Molecular analyses are presented in Table 5.

Spray Tanker Application

Aerosol samples collected from sites 1, 2, 3 in southern Arizona, all demonstrated concentrations of indicator microbes at or below detectable levels. Samples were collected between two and 20 m downwind of liquid biosolids application. Overall HPC bacteria were detected at levels greater than background concentrations, approximately 0.5 $\log_{10}$ greater, which was statistically significant ($P < 0.05$). At distances of 20 m HPC aerosol concentrations were statistically similar to background samples. Total coliform, *C. pefringens*, and *E. coli* were detected upon occasion, but were not detected with any frequency and only at distances within 5 m downwind of the operation. No aerosolized coliphage was detected. No pathogenic enteric viruses were detected via RTPCR.

Spray Irrigation

Site 9 consisted of aerosol samples collected downwind of 2 % liquid biosolids spray irrigation. All samples contained concentrations of HPC bacteria greater than most background samples collected, approximately 0.5 $\log_{10}$ greater. *C. perfringens*, total coliforms, and coliphage were detected at distances of 11 m and 40 m. This was the greatest distance from the application site that coliphage had been detected throughout this study. The detection of these indicator microbes was inconsistent as only a few samples were positive. Pathogenic viruses were not present through the use of RTPCR.

Cake 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

country. Through this operation, samples were collected from loading, slinging, spreading, unloading, background, and post processes.

Cake Spreading

Sites 5 and 10 consisted of aerosols collected from operations in which cake biosolids were spread via modified manure spreaders. Site 5 samples were collected downwind of loading sites, unloading sites, and spreading sites, whereas site 10 samples were collected two days post application of biosolids. Site 5 HPC concentrations from loading processes were statistically elevated over that of background, unloading, and spreading samples ($P < 0.05$). Total coliforms, *E. coli*, and *C. perfringens* were all detected during loading processes. Overall total coliforms were detected with in all samples collected from loading sites at distances between 2 m and 15 m, although concentrations decreased by two $\log_{10}$ to 10^2 MPN m^{-3} at 15 m ($P < 0.05$). Similar results were obtained from *E. coli* aerosol concentrations downwind of loading situations. *C. perfringens* was detected at minimal concentrations from loading, often barely above detection limits. Unloading events yielded *C. perfringens* upon one occasion while no other indicator microorganisms 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* only upon one occasion, while HPC bacteria were detected at approximately 1/2 $\log_{10}$ greater than background HPC concentrations. HPC concentrations decreased to levels similar to background concentrations ($P<0.05$) 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 days 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 Slinging

Sites 4, 6, 7, and 8 involved sample collection from biosolids land application 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 statistically significant. HPC bacteria concentrations were greater than background concentrations, and often times were 2 orders of magnitude greater than background levels, although this was not found to be statistically significant. However HPC aerosol concentrations involved with loading scenarios were significantly greater than slinging samples. Slinging samples 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 had positive PCR samples, two of which were detected during "slinging" samples and one collected during loading samples at 5 m and 2 m respectivley. 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 microorganisms 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 to loading and slinging situations.

Samples for site 7 were collected from a biosolids slinging operation consisting of loading and slinging samples. HPC concentrations during slinging operations were similar to background levels, while loading conditions yielded statistically significant ($P < 0.05$) levels approximately 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 concentrations. 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 modeling was performed utilizing a previously described transport model (Brooks et al. 2004). Although this model describes transport of coliphage from land applied biosolids, the model was also utilized here to describe bacterial transport. This approach is inherently conservative as aerosolized bacteria, specifically gram negative bacteria exhibit inactivation at a much greater rate than coliphage and hence travel less distance. (Brooks et al 2004). This

model was used to describe coliform and coliphage transport from land applied "cake" biosolids, specifically during loading and spreading operations.

To model coliform bacteria from loading operations, total coliform aerosol concentrations from loading operations (site 5, 2 m samples) were modeled with inactivation rates (0.036 [Log_{10} PFU m^{-3}] m^{-1} traveled) identical to the previously modeled coliphage (Brooks et al. 2004). To model coliform bacteria from spreading operations, detection limits (18.4 MPN m^{-3}, 1.26 Log_{10} MPN m^{-3}) during spreading operations were modeled in a similar fashion as the loading operations. 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, coliphage (site 8, 2 m samples) and *C. perfringens* (site 5, 2 m samples) were utilized respectively as stated above. In the latter case, *C. perfringens* concentrations could simulate coliphage concentrations as *C. pefringens*, a spore former, may better mimic the survival of coliphage.

Once indicator bacteria and coliphage were modeled, ratios were applied to estimate enteric pathogenic bacteria and viruses as previously described (Brooks et al 2004). A ratio of 1:10,000 (pathogenic bacteria/virus to indicator bacteria/virus) was used to predict aerosolized *Salmonella* spp. and coxsackievirus A21. This assumes that both will aerosolize with the same efficiency as the modeled predictions of aerosolized coliphage. This generated a microbial concentration, "x", at specific distances downwind of a biosolids operation. Risk of infection modeling was performed using the one-hit

exponential model (Haas et al. 1999), $p_i = 1 - \exp(-rN)$, and β-poisson infectivity model (Haas et al. 1999), $p_i = 1 - ((1 + N/N_{50})(2^{1/\alpha} - 1))^{-\alpha}$ where:

'r' = 0.0253 Coxsackievirus A21 (Couch et al. 1965),

'α' = 0.3126 *Salmonella* spp. (non-typhoid) (Haas et al. 1999)

'N_{50}' = 23,600 *Salmonella* spp. (Haas et al. 1999)

'N' = the exposure dose in number of organisms,

These models were chosen, as they most accurately describe the dose response to a one time coxsackievirus A21 and *Salmonella* spp aerosol exposure.

The exposure dose is described as, $N = x * 0.83 * t$, where:

'x' = the number of organisms per m^3

0.83 m^3 h^{-1} = the average human breathing rate (Environmental Protection Agency, 1997)

't' = the exposure duration in h

For *Salmonella* spp. exposures it was assumed that 10 % of inhaled microorganisms were also subsequently ingested (Medema et al. 2004).

The annual risk model is described as, $p_{(annual)} = 1 - (1 - p_i)^d$, where

'p_i' = the one time probability of infection, described above

'd' = the number of days exposed per year

Community Risk Analyses

Community exposure was described as any distance beyond 30.5 m downwind of an application site, as this represents the minimum setback distance from land application

site and a residential exposure (National Research Council 2002). Residences from sites 1 – 10, were located at least 500 m downwind from the land application site.

In this approach, loading scenario exposures present the greatest amount of exposure to bioaerosols. Risks of infection are shown in Table 6. One time risk of infection due to exposure to aerosolized *Salmonella* spp. from this operation results in a probability of infection of 5.67×10^{-7} when exposure occurs for one hour at least 30.5 m downwind from the site. Similarly, an eight hour exposure results in a 4.53×10^{-6} risk of infection. Annual risks of infection based on 1 and 8 hr exposures each day over 6 days year^{-1} resulted in 3.40×10^{-6} and 2.72×10^{-5} respectively. A 6 days per year exposure is assumed to be from two, three day biosolids application exposures per year (Brooks et al. 2004). Exposure to aerosolized coxsackievirus A21 during loading conditions for 1 and 8 hours exposures resulted in 7.85×10^{-6} and 6.28×10^{-5} respectively. Annual risks of infection resulted in 4.71×10^{-5} and 3.77×10^{-4} respectively.

During biosolids spreading operations, a one hour exposure to aerosolized *Salmonella* spp. results in 1.96×10^{-10}, while an eight hour exposure results in 1.57×10^{-9}. Annual risks of infection based on these same daily exposures and 6 days annually resulted in annual risks of 1.18×10^{-9} and 9.43×10^{-9} respectively. One hour and eight hour exposures to aerosolized coxsackievirus A21 resulted in 1.05×10^{-6} and 8.40×10^{-6} respectively. Annual risks of infection from one and eight hour exposures, 6 days per year resulted in 6.30×10^{-6} and 5.04×10^{-5} respectively.

DISCUSSION

Indicator microorganisms such as total coliforms, *E. coli*, *C. perfringens*, and coliphage were rarely detected, and detected concentrations were usually only slightly above detection limits. Only during biosolids loading operations did total coliforms and *E. coli* regularly reach levels above detection limits. Typically total coliforms and *E. coli* were only detected at distances within 15 m. One note of interest was the increased detection of indicator bacteria during sites with loading operations that also incorporated some soil, specifically sites 4 and 5. In this case soil particles may be protective against environmental inactivation factors such as dessication, ultraviolet light, and oxygen radicals (Lighthart, B., and Stetzenbach, L.D. 1994).

C. perfringens was more readily detected during all situations, but once again detection was limited to distances within 15 m. HPC bacteria was detected readily with the exception of sites located in areas of high relative humidity where soils were moist, such as site 6 and 8. Overall during biosolids operations, HPC bacteria were 1 $\log_{10}$ greater than background concentrations (10^3 HPC m^{-3}) and were regularly found at greater concentrations (> 2 $\log_{10}$) than any one specific biosolids borne microbe. In addition, HPC bacteria were not readily detected when soil was not incorporated into the biosolids loading (site 6, 8), i.e. soil was not collected along with the biosolids and hence mixed in with biosolids during front end loader operation. This limited aerosolized HPC concentrations to background concentrations, and hence leads to the hypothesis that the majority of HPC bacteria and consequently the majority of aerosolized microorganisms aerosolized during land application of biosolids are soil borne. Further investigations

into this phenomenon appear warranted. Although norovirus genomic material was detected upon three occasions via RT-PCR, it is unknown whether these were infectious viruses as no culturable system is available for this virus.

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 coliphages above detection limits possibly due to the particle size creation. This is speculated to be due to spray tankers, which generate dense liquid droplets of biosolids that could fall to the ground quickly upon aerosolization, limiting the opportunity for aerosolization of biosolids borne microorganisms.

On the other hand land application of liquid biosolids through the use of irrigators generated smaller less dense droplets, leading to detection of *C. perfringens*, total coliforms, and coliphage from distances of 11 m to 40 m respectively, downwind of the site, although neither was detected with frequency. It is important to reiterate that both processes of liquid biosolids application are rarely used throughout the country based on field observations.

Overall community microbial risk of infection associated with land application of biosolids, specifically "cake" application is minimal based on this study. Using conservative transport modeling approaches (ie. the use of a model generated by coliphage transport) to model bacteria increases the calculated risk of infection. Although indicators were rarely detected and with little frequency, in this conservative

approach the average of detected aerosolized indicators during loading and spreading conditions were used to estimate transport of pathogens.

One-time risks of infection associated with *Salmonella* spp, at distances greater than 30.5 m were minimal, although risk of infection from *Salmonella* is significant at 30.5 m downwind of loading operations. Annual risks of infection proved to be significant at 30.5 m downwind of loading operations, based on conservative assumptions, 8 hour exposures per day. These assumptions may 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 10% of all inhaled bacteria are also subsequently swallowed, a conservative assumption. Another important point to state is that loading operations typically are not situations in which community exposure would be significant because of their short duration. Spreading situations would appear to present little risk of infection, both from one time and annual, as these are moving point sources, and little time is spent at one specific location on site. Hence exposure would be very limited at a fixed location, i.e. a single residence.

One-time viral risks of infection at 30.5 m are significant, while at greater distances, risks are insignificant from loading situations. Although it is important to point out that, loading situations as stated above, are overestimates of the risk of infection as these exposures are of short duration and are typically located at one on site location. While these viral risks are based on coxsackievirus A21, these calculations may overestimate the risk of infection as the concentrations of coxsackievirus A21 present in biosolids may not be that significant, however these viral risks do not represent risk from other known

enteric viruses. Spreading operations present minimal risks based on these assumptions and analyses. Similar one time and annual risks of infection from exposure to Coxsackievirus A21 during land application of liquid biosolids was calculated using a modeling approach, in a previous study (Brooks et al 2004). This previous study determined that at 30.5 m downwind of the source, risks of infection were approximately 1 order of magnitude less than values presented here for loading operations.

Overall from this current study, risks of infection from one time and annual exposure calculations proved to be minimal even at distances within 30.5 m downwind of the source based on these conservative approaches. Most notably loading situations proved to be the greatest risk as these sources were overestimates of the actual risk for reasons stated previously. It is important to note that while this study assumes 30.5 m to be community risk, most communities would be located at greater distances.

ACKNOWLEDGEMENTS

This work was supported by funding from the University of Arizona National Science Foundation Water Quality Center.

Table 1 Sample sites throughout the continental USA and associated biosolids application method and aerosol samples collected.

Site	Sample type	Dist (m)	Location	Collection Dates	RH %	Temp C	WS m/s	# of Samples Collected	Type of Biosolids	Application Method	Aerosol Samples Collected
1a 1b 1c	DW Spray Trk DW Spray Trk BG	2 20 BG	Marana, Az	2/8/02 - 2/19/03	20.0	16.0	2.1	47	Class B Anaeobic Liquid (7-8%)	Spray Tanker Betterbuilt	DW - Spray Application Moving Point Source BG
2a 2b	DW Spray Trk BG	2 BG	Eloy, Az	3/21/02 - 6/6/02	15.6	21.8	1.5	24	Class B Anaeobic Liquid (7-8%)	Spray Tanker Betterbuilt	DW - Spray Application Moving Point Source BG
3a 3b	DW Spray Trk BG	2 BG	Picacho, Az	6/19/2002	11.5	25.2	1.5	6	Class B Anaeobic Liquid (7-8%)	Spray Tanker Betterbuilt	DW - Spray Application Moving Pt Src BG
4a 4b 4c 4d 4e 4f	DW Slinging DW Slinging DW Loading DW Loading DW Loading BG	2 5 2 5 10 BG	Mojave, Az	7/16/02 - 7/19/02	37.5	34.3	1.1	48	Class B Anaeobic Cake (21%)	Slinger Knight Protwin Slinger	DW - Loading -Stationary Pt Src DW - Slinging -Moving Pt Src BG
5a 5b 5c 5d 5e 5f 5g 5h	DW Spreading DW Spreading DW Spreading DW Loading DW Loading DW Unloading DW Unloading BG	2 – 21 18 – 28 25 – 37 2 15 10 13 BG	Solano, Ca	8/6/02 - 8/8/02	40.4	22.1	2.5	45	Class B Anaeobic Cake (20%)	Manure Spreader	DW - Loading- Stationary Pt Src DW - Spreading - Moving Pt Src DW - Unloadin - Stationary Pt Src BG
6a 6b 6c 6d 6e	DW Slinging DW Slinging DW Loading DW Loading BG	2 10 2 5 BG	Snoqualamie, Wa	1/13/03 - 1/15/03	75.6	8.0	0.3	42	Class B Anaeobic Cake (16%)	Slinger Aerosopread	DW - Loading - Stationary Pt Src DW - Slinging - Moving Pt Src BG
7a 7b 7c 7d 7e	DW Slinging DW Slinging DW Loading DW Loading BG	2 5 2 20 BG	Sunnyside, Wa	3/25/03 - 3/27/03	41.4	13.8	2.1	43	Class B Anaeobic Cake (27.6%)	Slinger Knight Protwin Slinger	DW - Loading - Stationary Pt Src DW - Slinging - Moving Pt Src BG
8a 8b 8c	DW Loading DW Loading BG	2 20 – 30 BG	Leesburg, Va	5/6/03 - 5/7/03	54.3	18.5	0.7	36	Class B Anaeobic Cake (24%)	Slinger Knight Protwin Slinger	DW - Loading - Stationary Pt Src BG
9a 9b	DW Spray Irr DW Spray Irr	11 37.5 – 50	Houston, Tx	8/6/03 - 8/7/03	39.8	36.5	2.3	30	Class B Anaeobic Liquid (2%)	Spray Irrigation	DW - Irrigation - Stationary Pt Src
10a	Post	Post	Chicago, Il	8/20/2003	54.4	19.8	1.6	40	Class B Anaeobic Cake (17%)	Spreader AgChem TerraGator	DW - Post - Startionary Area Src

* DW – Downwind, BG – Background, Dist – Distance, Pt Src – Point Source

RH - Relative humidity, Temp – Temperature, WS - Windspeed

Table 2 Biosolids microbial concentrations from sample sites throughout the country.

Site	HPC	TotalColiform	*E.coli*	Coliphage	*C. perfringens*
	CFU/g	MPN/g	MPN/g	PFU/g	CFU/g
1--1	2.74×10^{8}	1.27×10^{5}	5.43×10^{4}	NoData	4.34×10^{6}
1--2	4.15×10^{8}	1.34×10^{5}	2.87×10^{4}	1.17×10^{4}	4.80×10^{6}
1--3	4.21×10^{8}	8.33×10^{5}	4.34×10^{4}	3.02×10^{4}	2.39×10^{6}
2--1	3.53×10^{8}	1.75×10^{5}	1.17×10^{4}	1.45×10^{4}	3.83×10^{6}
2--2	3.35×10^{8}	3.23×10^{5}	5.44×10^{4}	1.71×10^{4}	3.55×10^{6}
2--3	5.91×10^{8}	8.94×10^{4}	1.26×10^{4}	1.09×10^{4}	2.16×10^{6}
4	6.14×10^{8}	9.67×10^{5}	1.95×10^{5}	NoData	9.35×10^{5}
5	5.40×10^{9}	4.33×10^{8}	3.85×10^{8}	NoData	7.00×10^{5}
6	2.55×10^{7}	1.37×10^{5}	1.20×10^{4}	8.72×10^{3}	3.75×10^{5}
7	1.69×10^{10}	4.63×10^{5}	1.75×10^{5}	2.84×10^{3}	4.58×10^{5}
8	Nodata	Nodata	Nodata	NoData	Nodata
9	5.20×10^{6}	4.10×10^{1}	4.00×10^{0}	7.00×10^{0}	3.84×10^{5}
10	1.38×10^{8}	1.48×10^{6}	1.08×10^{5}	1.03×10^{3}	1.01×10^{5}

* Biosolids from sites 1, 2, and 3 were all from the same biosolids treatment plant and hence only sample sites 1 and 2 are noted.

* No data represents lack of sample or sample loss.

Table 3 Frequency and percentage of aerosol samples positive for assayed microbes.

Site	HPC Frequency	HPC %	TotCol Frequency	TotCol %	*E.coli* Frequency	*E. coli* %	*C perfringens* Frequency	*C. perfringens* %	*Coliphage* Frequency	Coliphage %
1a	25\25	100	3\25	12	2\25	8	4\25	16	0\13	0
1b	4\4	100	0\4	0	0\4	0	0\4	0	0\4	0
1c	2\2	100	0\2	0	0\2	0	0\2	0	0\2	0
2a	19\19	100	2\19	11	0\19	0	1\19	5	3\19	16
2b	5\5	100	0\5	0	0\5	0	0\5	0	0\5	0
3a	4\4	100	0\4	0	0\4	0	1\4	25	0\4	0
3b	2\2	100	0\2	0	0\2	0	0\2	0	0\2	0
4a	7\7	100	0\7	0	0\7	0	0\7	0	0\7	0
4b	15\15	100	0\15	0	0\15	0	0\15	0	0\15	0
4c	6\6	100	2\6	33	1\6	17	3\6	50	0\6	0
4d	3\3	100	1\3	33	0\3	0	1\3	33	0\3	0
4e	3\3	100	0\3	0	0\3	0	1\3	33	0\3	0
4f	2\2	100	0\2	0	0\2	0	0\2	0	0\2	0
5a	6\6	100	0\6	0	0\6	0	1\9	11	0\6	0
5b	6\6	100	0\6	0	0\6	0	0\6	0	0\6	0
5c	6\6	100	0\6	0	0\6	0	0\6	0	0\6	0
5d	6\6	100	6\6	100	6\6	100	3\6	50	0\6	0
5e	6\6	100	6\6	100	5\6	83	4\6	67	0\6	0
5f	6\6	100	0\6	0	0\6	0	0\6	0	0\6	0
5g	6\6	100	0\6	0	0\6	0	1\6	17	0\6	0
5h	3\3	100	0\3	0	0\3	0	0\3	0	0\3	0
6a	5\6	83	0\6	0	0\6	0	0\6	0	0\6	0
6b	5\6	83	0\6	0	0\6	0	0\6	0	0\6	0
6c	7\12	58	0\12	0	0\12	0	0\12	0	0\12	0
6d	5\12	42	0\12	0	0\12	0	0\12	0	0\12	0
6e	4\6	67	0\6	0	0\6	0	0\6	0	0\6	0
7a	6\6	100	0\6	0	0\6	0	0\6	0	0\6	0
7b	4\4	100	0\4	0	0\4	0	0\4	0	0\4	0
7c	10\10	100	0\10	0	0\10	0	0\10	0	0\10	0
7d	12\12	100	1\12	8	0\12	0	0\12	0	1\12	8
7e	5\5	100	0\5	0	0\5	0	0\5	0	0\5	0
8a	7\9	71	0\9	0	0\9	0	0\9	0	1\9	11
8b	9\9	100	0\9	0	0\9	0	0\9	0	2\9	22
8c	10\12	83	0\12	0	0\12	0	0\12	0	0\12	0
9a	6\6	100	1\6	17	0\6	0	1\6	17	0\6	0
9b	17\17	100	1\18	6	0\18	0	0\18	0	1\18	6
10a	39\39	100	0\39	0	0\39	0	0\39	0	0\39	0

* HPC – Heterotrophic Plate Count bacteria, TotCol – Total Coliforms

Table 4 Detected aerosol microbial concentrations and ranges for each microbe assayed.

Site	HPC Range	HPC avg	TotCol Range	TotCol Avg	*E coli* Range	*E coli* Avg	*C perfringens* Range	*C perfringens* Avg	Coliphage Range	Coliphage Avg
	CFU m^{-3}		MPN m^{-3}		MPN m^{-3}		CFU m^{-3}		PFU m^{-3}	
1a	4.91×10^{3} - 2.24×10^{5}	4.55×10^{4}	BD - 9.00×10^{3}	4.70×10^{2}	BD - 6.77×10^{3}	3.02×10^{2}	BD - 8.85×10^{1}	7.77×10^{0}	BD	BD
1b	3.68×10^{3} - 6.75×10^{3}	5.45×10^{3}	BD	BD	BD	BD	BD	BD	BD	BD
1c	6.48×10^{3} - 3.07×10^{4}	1.86×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
2a	7.05×10^{3} - 2.13×10^{5}	7.11×10^{4}	BD - 1.23×10^{1}	1.04×10^{0}	BD	BD	BD - 1.02×10^{1}	6.38×10^{-1}	BD - 1.84×10^{2}	1.56×10^{1}
2b	8.71×10^{2} - 2.07×10^{5}	1.35×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
3a	1.47×10^{4} - 2.86×10^{4}	2.03×10^{4}	BD	BD	BD	BD	BD - 4.42×10^{1}	1.11×10^{1}	BD	BD
3b	8.48×10^{3} - 1.6×10^{4}	1.22×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
4a	5.52×10^{3} - 1.92×10^{4}	1.01×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
4b	2.76×10^{3} - 4.66×10^{5}	8.71×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
4c	1.24×10^{4} - 1.03×10^{8}	1.60×10^{7}	BD - 8.43×10^{2}	2.00×10^{2}	BD - 2.96×10^{2}	4.93×10^{1}	BD - 2.94×10^{1}	6.13×10^{0}	BD	BD
4d	1.01×10^{4} - 1.61×10^{6}	4.71×10^{5}	BD - 1.47×10^{1}	4.9×10^{0}	BD	BD	BD - 1.47×10^{1}	2.45×10^{0}	BD	BD
4e	1.09×10^{4} - 2.33×10^{6}	5.58×10^{5}	BD - 6.76×10^{2}	2.25×10^{2}	BD	BD	BD - 1.47×10^{1}	2.45×10^{0}	BD	BD
4f	3.37×10^{3} - 7.2×10^{3}	5.29×10^{3}	BD	BD	BD	BD	BD	BD	BD	BD
5a	2.43×10^{4} - 4.71×10^{5}	1.03×10^{5}	BD	BD	BD	BD	BD - 1.64×10^{1}	2.73×10^{0}	BD	BD
5b	3.56×10^{4} - 6.59×10^{4}	5.34×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
5c	7.05×10^{3} - 2.36×10^{4}	1.41×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
5d	1.70×10^{5} - 8.12×10	4.89×10^{5}	1.55×10^{2} - 2.48×10^{4}	7.63×10^{3}	1.28×10^{2} - 1.48×10^{4}	3.16×10^{3}	BD - 2.45×10^{1}	8.45×10^{0}	BD	BD
5e	1.14×10^{5} - 1.04×10^{6}	3.56×10^{5}	2.45×10^{1} - 6.28×10^{2}	1.57×10^{2}	BD - 2.77×10^{2}	7.73×10^{1}	BD - 2.45×10^{1}	1.23×10^{1}	BD	BD
5f	9.81×10^{3} - 2.18×10^{4}	1.51×10^{4}	BD - BD	BD	BD	BD	BD	BD	BD	BD
5g	7.82×10^{3} - 1.33×10^{4}	1.05×10^{4}	BD - BD	BD	BD	BD	BD - 1.64×10^{1}	2.73×10^{0}	BD	BD
5h	1.38×10^{4} - 2.37×10	2.00×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
6a	BD - 3.07×10^{3}	1.10×10^{3}	BD	BD	BD	BD	BD	BD	BD	BD
6b	BD - 2.30×10^{3}	7.92×10^{2}	BD	BD	BD	BD	BD	BD	BD	BD
6c	BD - 2.76×10^{3}	5.24×10^{2}	BD	BD	BD	BD	BD	BD	BD	BD
6d	BD - 1.23×10^{3}	2.43×10^{2}	BD	BD	BD	BD	BD	BD	BD	BD
6e	BD - 9.20×10^{2}	2.81×10^{2}	BD	BD	BD	BD	BD	BD	BD	BD
7a	2.24×10^{4} - 9.02×10^{4}	4.19×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
7b	1.63×10^{4} - 9.29×10^{4}	4.17×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
7c	3.53×10^{4} - 3.13×10^{5}	1.45×10^{5}	BD	BD	BD	BD	BD	BD	BD	BD
7d	5.34×10^{4} - 5.37×10^{5}	1.91×10^{5}	BD - 3.68×10^{1}	3.07×10^{0}	BD	BD	BD	BD	BD - 9.2×10^{1}	7.67×10^{0}
7e	1.93×10^{4} - 8.49×10^{4}	4.18×10^{4}	BD	BD	BD	BD	BD	BD	BD	BD
8a	BD - 1.20×10^{4}	3.76×10^{3}	BD	BD	BD	BD	BD	BD	BD - 3.07×10^{1}	3.41×10^{0}
8b	6.16×10^{2} - 3.68×10^{3}	1.77×10^{3}	BD	BD	BD	BD	BD	BD	BD - 1.84×10^{2}	2.39×10^{1}
8c	BD - 1.35×10^{4}	1.79×10^{3}	BD	BD	BD	BD	BD	BD	BD	BD
9a	4.09×10^{3} - 2.27×10	3.16×10^{4}	BD	BD - 1.84×10^{1}	BD	BD	BD - 1.84×10^{1}	3.07×10^{0}	BD	BD
9b	1.26×10^{4} - 1.35×10^{5}	4.41×10^{4}	BD	BD - 1.84×10^{1}	BD	BD	BD	BD	BD - 2.30×10^{1}	1.35×10^{0}
10a	3.04×10^{2} - 5.06×10^{4}	3.36×10^{3}	BD	BD	BD	BD	BD	BD	BD	BD

* HPC – Heterotrophic Plate Count bacteria, TotCol – Total Coliforms

*Detection Limits – HPC 307 CFU m^{-3}, TotCol 18 MPN m^{-3}, *E. coli* 18 MPN m^{-3}, *C. perfringens* 18 CFU m^{-3}, and coliphage 23 PFU m^{-3}.

Table 5 RTPCR primer sequences, amplicons, and number of samples positive for each virus screened.

Organism	Primer/Sequence	Amplicon Size/Region	Number of Samples	Number of Positive	Det Limit copies/m^3
NLV	RegA - CTCRTCATCICCATARAAIGA MJV12 - TAYCAYTATGATGCHGAYTA	327 bp - Region A POL gene	315	3	34 - 99
Enterovirus	P1 - CCTCCGGCCCCTGAATG P2 - ACCGGATGGCCAATCCAA ent33 - CCCAAAGTAGTCGGTTCCGC	197 bp - conserved 5' untranslated region 105 bp - internal amplicon	315	0	34 - 99
HAV	H1 - CAGCACATCATCAGAAAGGTGAG H2 - CTCCAGAATCATCTCCAC	192 bp - capsid protein	315	0	34 - 99

* Detection limits based on two total sample volumes assayed, 10 L and 29 L.

* Three samples were determined to contain norovirus derived nucleic acid, two samples 5 m downwind of slinging operations, and one sample 2 m downwind of loading operations.

Table 6 Probabilities of infection for *Salmonella* (non typhoid) and coxsackievirus A21 downwind of loading and spreading operations.

	One time Risks of Infection							
	Spreading Operations				Loading Operations			
	Salmonella spp.		Coxsackievirus A21		*Salmonella spp.*		Coxsackievirus A21	
DW Distance (m)	Exposure time				Exposure time			
	1 hr	8 hr	1 hr	8 hr	1 hr	8 hr	1 hr	8r
30.5	1.96×10^{-10}	1.57×10^{-9}	1.05×10^{-6}	8.40×10^{-6}	5.67×10^{-7}	4.53×10^{-6}	7.85×10^{-6}	6.28×10^{-5}
50	3.82×10^{-11}	3.06×10^{-10}	2.04×10^{-7}	1.63×10^{-6}	1.10×10^{-7}	8.82×10^{-7}	1.53×10^{-6}	1.22×10^{-5}
83.9	2.22×10^{-12}	1.78×10^{-11}	1.19×10^{-8}	9.50×10^{-8}	6.41×10^{-9}	5.13×10^{-8}	8.88×10^{-8}	7.11×10^{-7}
100	5.75×10^{-13}	4.60×10^{-12}	3.08×10^{-9}	2.46×10^{-8}	1.66×10^{-9}	1.33×10^{-8}	2.30×10^{-8}	1.84×10^{-7}
500	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0
	Annual Risks of Infection							
	Spreading Operations				Loading Operations			
	Salmonella spp.		Coxsackievirus A21		*Salmonella spp.*		Coxsackievirus A21	
	Exposure time				Exposure time			
	1 hr	8 hr	1 hr	8 hr	1 hr	8 hr	1 hr	8r
30.5	1.18×10^{-9}	9.43×10^{-9}	6.29×10^{-6}	5.04×10^{-5}	3.40×10^{-6}	2.72×10^{-5}	4.71×10^{-5}	3.77×10^{-4}
50	2.29×10^{-10}	1.83×10^{-9}	1.22×10^{-6}	9.81×10^{-6}	6.62×10^{-7}	5.29×10^{-6}	9.17×10^{-6}	7.34×10^{-5}
83.9	1.33×10^{-11}	1.07×10^{-10}	7.13×10^{-8}	5.70×10^{-7}	3.85×10^{-8}	3.08×10^{-7}	5.33×10^{-7}	4.26×10^{-6}
100	345×10^{-12}	2.76×10^{-11}	1.85×10^{-8}	1.48×10^{-7}	9.96×10^{-9}	7.97×10^{-8}	1.38×10^{-7}	1.10×10^{-6}
500	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0

* Annual risks of infection based on 6 days exposure per year.

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APPENDIX D

The Occurrence of Aerosolized Endotoxin from Land Application of Class B Biosolids[#]

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[#] To be submitted to the Journal of Applied Microbiology (2004)

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SUMMARY

AIM: The purpose of this study was to determine aerosolized endotoxin concentrations downwind of a biosolids land application site.

METHODS AND RESULTS: Aerosol samples were collected from biosolids land application sites, tractor operation, and an aeration basin located within an open- air wastewater treatment plant. Aerosolized endotoxin above background concentrations was detected from all sites, at levels ranging from below detection to 1800 EU m^{-3}. Biosolids loading operations resulted in the greatest concentrations of endotoxin (mean 344 EU m^{-3}). As downwind distance increased from sources, levels of endotoxin decreased to near background concentrations.

CONCLUSIONS: Overall, the levels of aerosolized endotoxin were within limits (1000 EU m^{-3}) proposed by other occupational exposure studies, and were only occasionally found above these limits. Sites in which soil was being aerosolized resulted in greater concentrations of endotoxin with or without biosolids, which suggested that the majority of endotoxin may in fact be soil borne.

SIGNIFICANCE AND IMPACT OF STUDY: This study measured the presence of aerosolized endotoxin from the land application of biosolids, and showed that these levels were within acceptable limits with respect to occupational exposures.

Keywords: biosolids, sludge, pathogen, risk, aerosol, coliphage

INTRODUCTION

Throughout the United States, it has been estimated that approximately 6.5 million tons of biosolids are produced and that 60% of this is land applied (National Research Council 2002). Class B biosolids are land applied in rural areas, but with the increased growth of urban areas, the transport of biosolids to rural areas is becoming increasingly more difficult. Although pathogenic microorganisms such as, *Salmonella, Escherichia coli*, *Ascaris* ova, and enteric viruses have received the most attention, little attention has been focused on bacterial endotoxin.

Endotoxin, or lipopolysaccharide (LPS) derived from the cell wall of gram negative bacteria is a highly immunogenic molecule, that when introduced directly into the bloodstream has demonstrated the ability to cause a broad range of health effects such as fever, asthma, and shock (hence the suffix "toxin") (Bradley, S.G. 1979; Olenchock, S.A. 2001; Michel, O. 2003). Lipopolysaccharide is present ubiquitously throughout the environment, as gram negative bacteria continuously release LPS during both cell decay and active cell growth. Most surfaces contain some traces of endotoxin due to dust associated endotoxin, and therefore most human populations come into contact with some endotoxin (Gereda et al. 2001; Sharif et al. 2004). Although endotoxin is present in "everyday" environments, it is primarily of concern as an aerosol, since most ailments are pulmonary associated.

Exposures to aerosolized endotoxin have been specifically studied regarding occupational exposures from cotton dust, composting plants, and feed houses (Clark et al. 1983; Rylander et al. 1983; Castellan et al. 1987; Smid et al. 1992; Epstein, E. 1994;

Donham et al. 2000). Exposures to levels of endotoxin as little as 0.2 endotoxin unit (EU) m^{-3} derived from poultry dust have been found to cause acute pulmonary ailments such as decreases in forced expiratory volume (FEV) (Donham et al. 2000). Chronic effects such as asthma and chronic bronchitis have been found to be due to exposures of endotoxin from cotton dust as little as 10 EU m^{-3} on a daily basis (Olenchock, S.A. 2001).

Past studies that have been conducted regarding environmental exposures to endotoxin, have used methods such as membrane trapping of aerosolized endotoxin. Recently a study compared methods of aerosolized endotoxin collection between traditional membrane trappings and collection via impingement (Duchaine et al. 2001). Results suggest differences between the two methods, and that impingement may result in higher percent recoveries and greater precision. This same study focused on aerosolized endotoxin exposure in occupational settings, specifically swine barns and sawmills. It was shown that swine barns were found to contain mean concentrations of endotoxin ten times greater than that of sawmills, 4,385 and 740 EU m^{-3} respectively. Endotoxin concentration ranged from a minimum of 208 to 17,063 EU m^{-3} for sawmills, and from 2,026 to 11, 297 EU m^{-3} for swine barns as collected by impingement sampling.

Composting sites have also been studied with respect to endotoxin exposures, and although most sites have been shown to contain concentrations of aerosolized endotoxin greater than that of background levels, these levels were thought to be within safe limits, < 1000 EU m^{-3} (Rylander et al. 1983). It was suggested by the authors that 1000 EU m^{-3} should be considered safe with regard to human health until additional studies have been conducted. A study conducted by Clark et al (1983) determined aerosolized endotoxin

concentrations from a composting plant to be between 10 to 400 EU m^{-3}. It is important to note that despite the presence of endotoxin within these sites, there was no evidence of residential impact, since beyond the composting site boundaries levels regressed to background concentrations.

No studies to date have been conducted regarding aerosolized endotoxin exposure from the land application of biosolids. Class B biosolids contain coliform concentrations upwards of 10^6 g^{-1} and theoretically could harbor high levels of endotoxin (Brooks et al. 2004). Therefore land application of Class B biosolids may contribute to aerosolized endotoxin. Hence this study focused on the generation of aerosolized endotoxin from the land application of biosolids, and compared these exposures to other environments conducive to the generation of aerosolized endotoxin.

MATERIALS AND METHODS

Site and biosolid application operation

Land application sites receiving biosolids throughout southern Arizona were chosen for this study. Most land application took place on dry agricultural fields, typically used for the cultivation of cotton. All biosolids were mesophilically anaerobically digested 20 % dry mass "cake" Class B biosolids originating from Maricopa County, AZ, and were land applied via the use of a Knight Protwin slinger (Kuhn Knight Inc; Brodhead, WI). Samples were collected downwind of "loading", "slinging", and "total operation" at multiple downwind distances from the source (Table 1). "Loading" samples are defined here as aerosol samples collected downwind of slinger loading using a front-end loader. "Slinging" samples are defined here as downwind aerosol samples collected from the

operation of a Knight Protwin slinger, during which biosolids are launched into the air approximately 15 m. "Total operation" samples are defined as aerosol samples collected downwind of the entire operation, typically at a location bordering the operation site. These samples were collected at a location in which sampled air could not be attributed to "loading" or "slinging" situations. Samples were collected from 4/2004 to 6/2004.

Non-biosolids application sites

In addition to aerosol samples collected from biosolids land application sites, samples were collected from other sites. Specifically, samples were collected from an open-air activated sludge wastewater treatment plant, an agricultural field during tractor operation, and an agricultural field where no biosolids were applied and no mechanical operations took place. The latter sample was designated as a "background" sample, used to assess the typical concentrations of endotoxin present in normal agricultural settings where cotton was cultivated. Wastewater treatment plant samples were collected 2 m downwind of an aeration basin utilizing bubble aeration within the Roger Road Wastewater Treatment Plant located in Tucson, Az.

Aerosol collection

Samples were collected via the use of three SKC Biosamplers® (SKC West Inc; Fullerton, CA) operating at an air intake rate of 12.5 L min^{-1}. Prior to use, all glassware was autoclaved and heated at 180° C for 3 hr to remove any endotoxin remnants, a step known as depyrogenation. Thoughout the study, samplers were randomly chosen to be tested for the presence of background endotoxin, to determine the effectiveness of the depyrogenation step. Samplers were placed upon aluminum tripods set at a height to

where the intake nozzle approximated the average human breathing height of 1.5 m (ASTM 1993). Air intake was provided through the use of SKC Vac-U-Go vacuum pumps (SKC West Inc; Fullerton, CA). Samplers were loaded with 23 mL of sterile non-pyrogenic injection water used for drug dilutions (Abott Laboratories, Chicago, IL). Samples were collected for 10 minutes downwind of each operation in triplicate at a specific downwind distance between 2 and 200 m as previously described (Brooks et al. 2004). Following collection, samples were aseptically removed from the sample basin and placed within sterile non-pyrogenic 50 mL polystyrene centrifuge tubes (Corning Inc; Corning, NY). Samples were then placed on ice and transported to the laboratory, and subsequently frozen at –20° C until further analysis. Prior to freezing, sample volumes were noted.

Endotoxin assay

All glassware used in the assay was depyrogenated prior to use at 180° C for 3 hr. The commercially available Pyrotell T® turbidmetric *Limulus* Amebocyte Lysate Assay (Associates of Cape Cod; E. Falmouth, MA) was employed for the detection of both bound and liberated endotoxin present within the aerosol samples. In the presence of endotoxin, *Limulus* amebocytes (Pyrotell T®) coagulates and results in the formation of turbidity.

Samples were defrosted at room temperature and subsequently vortexed (VWR Int; W. Chester, PA) for 2 min prior to extraction of an aliquot for assay. To perform the assay, samples were transferred to a sterile non-pyrogenic 96 well microtiter plate (Associates of Cape Cod; E. Falmouth, MA) using aerosol barrier micropipette tips. The

outer most 36 sample wells were left blank as recommended by the manufacturer. Samples and subsequent serial dilutions (0.1 mL aliquots) were loaded into the microtiter plate in duplicate. Following aliquot additions, 0.1 mL of *Limulus* amebocyte extract (Pyrotell T®) was aseptically added to each microtiter well containing sample aliquots. Subsequently the microtiter plate was manually mixed by repeated finger tapping of the edges of the plate for 30 seconds, effectively mixing each well. Plates were then placed on a pre-heated microtiter plate dry block incubation well (VWR Int; W. Chester, PA) set at 37 +/- 1° C, and incubated for 27 minutes.

Assay controls were also processed to assess the efficiency of the process as suggested by the manufacturer. Negative assay controls containing sterile non-pyrogenic water, and negative collection controls consisting of sterile non-pyrogenic water washed through the Biosamplers were both assayed. Negative controls were present in each set of samples assayed. Control standard endotoxin (CSE) (Associates of Cape Cod; E. Falmouth, MA) derived from *Escherichia coli* O113:H10 was utilized to develop a standard linear regression to which all sample absorbance readings were compared. The CSE linear regression was performed with each set of samples. CSE standard curves contained a range of endotoxin concentrations from 1.25 EU mL^{-1} to 0.0389 EU mL^{-1} in twofold serial dilutions. Positive product controls consisting of a spike concentration of 0.3125 EU mL^{-1} were added to multiple samples or dilutions of samples chosen at random to determine inhibition/enhancement of the endotoxin determination assay (Hollander et al. 1993). These controls were performed prior to sample collection and throughout each sampling period.

Following plate incubation, all plates were quickly removed from the incubator and placed on a BioTek ELX 808 microplate reader (BioTek Instruments Inc; Winooski, VT) able to read turbidity at an absorbance wavelength of 360 nm. Absorbance values for samples and dilutions were then compared to the standard linear regression and endotoxin concentrations were determined.

Statistical analyses

Analysis of variance and basic statistics were performed through the use of the Minitab statistical analysis program (Minitab Inc; State College, PA).

RESULTS

Controls - background

Samples collected from an agricultural field with no land application or agricultural activities yielded aerosolized endotoxin concentrations near detection limits. The mean sample concentration was 2.66 EU m^{-3} with a minimum of 2.33 and a maximum of 3.84 EU m^{-3}.

Biosolids land application sites

Sites undergoing land application of biosolids yielded elevated levels of endotoxin above background concentrations, ($P < 0.05$). Levels ranged from 4.9 EU m^{-3} to 1,808 EU m^{-3} (Table 2) (Figure 1). Loading events, sampled at 2 m, yielded the greatest mean concentration and maximum value, 543 and 1,808 EU m^{-3} respectively. At distances of 40 m downwind of the loading operations, endotoxin levels began to decrease to values close to background concentrations. Aerosol samples collected 10 m from biosolids slinging operations averaged 114 EU m^{-3}. Beyond 10 m, aerosolized endotoxin

concentrations decreased to a mean of approximately 6 EU m^{-3}. Samples downwind of the total operation, those with loading and slinging included, averaged 134 EU m^{-3}, and ranged from 6 to 624 EU m^{-3}. Samples collected from 2 m downwind of a biosolids pile yielded an average aerosolized endotoxin concentrations of 103 EU m^{-3}. All biosolids operations at all sampled downwind distances yielded aerosolized concentrations of endotoxin greater than that of background levels, ($P < 0.05$).

Non-biosolids sites

Air samples collected during tractor operations, in soil which had not received biosolids in at least 20 years, yielded endotoxin concentrations which were similar to those from biosolids loading sites, ($P > 0.05$). Aerosolized endotoxin concentrations ranged from 284 to 659 with an average of 469 EU m^{-3}. Aerosol samples collected from 2 m downwind of a wastewater treatment plant secondary aeration basin yielded endotoxin concentrations ranging from 294 to 891 EU m^{-3}. Aeration basin derived aerosols contained an endotoxin mean concentration of 627 EU m^{-3}, which did not differ from biosolids loading sites or tractor operation sites, ($P > 0.05$). All endotoxin concentrations were significantly greater than background samples, ($P < 0.05$).

DISCUSSION

Overall concentrations of aerosolized endotoxin from land applied biosolids did not reach levels previously thought of as levels of concern from other occupational exposures. On average, maximum levels were below the suggested safe level of endotoxin which is 1000 EU m^{-3}, as suggested by studies of swine confinement workers and sewage composting plants (Rylander et al 1983; Donham et al 1989). In addition aerosols

samples collected from downwind of total operation sites were found to contain endotoxin concentrations similar to that of loading operations, tractor operations, and aeration basins. This was believed to be a factor of the loading operation, as these samples were collected downwind of both loading and slinging sites simultaneously. Samples collected from downwind of loading sites are known to contain soil particles (dust) as soil is incorporated into the biosolids loading process through the action of the front-end loader. Thus soil itself could be contributing to the greater concentrations of endotoxin as well as overall aerosolized microbial concentrations as previously described (Brooks et al 2004). In addition, soil may also contribute to the majority of aerosolized endotoxin that results from land application operations. Based on recent research, soil in which no biosolids had been previously applied were found to contain about 10 EU mg^{-1} (unpublished data).

Samples located from downwind of aeration basins and downwind of tractor operations did contain endotoxin concentrations similar to that of biosolids loading sites, although maximum concentrations did not reach levels similar to that of loading sites. Typically, these levels were three times below that of the maximum endotoxin concentration measured from biosolids loading sites. Aerosol samples collected from downwind of a tractor operation bolster the assumption that soil contributes to the majority of aerosolized endotoxin from biosolids operations. It is important to note that aeration basin endotoxin concentration means were greater than tractor and loading concentrations. This is most likely due to the constant efflux of endotoxin emitted during the wastewater treatment process, as opposed to the periodical effluxes of endotoxin

emitted during the land application and tractor point sources. In addition the presence of elevated levels of endotoxin from a wastewater treatment plant is explained by the presence of high concentrations of gram-negative bacteria present in wastewater.

At downwind distances of up to and including 200 m from the biosolids application sources endotoxin concentrations did decline, although concentrations were still greater than background levels. While this may seem ominous it is important to note that communities exposed to these endotoxin concentrations as a result of biosolids operations, are in fact exposed to similar or greater levels of endotoxin by simply exposing themselves to a dusty road or environment. In addition not all endotoxin exposures are detrimental. It has been demonstrated that exposures to low levels of endotoxin in farming communities has resulted in members of the community with decreased immune responses to endotoxin, much like allergy desensitization (Braun-Fahrlander et al. 2002; Liu, A.H. and Redmon A.H. 2001; Kaiser H.B. 2004). Although much like in allergic rhinitis, some exposed will experience this type of reaction, while others may not experience this.

Studies on health effects have largely been demonstrated in indoor work environments where continuous exposure takes place. Dose response studies in occupational exposures such as poultry workers, has demonstrated that as little as a total endotoxin concentration of 0.240 EU m^{-3} is needed to induce a reduction in acute pulmonary function, although the threshold level suggested by the authors of the study for a significant reduction in pulmonary function was noted to be continuous exposure to a total endotoxin concentration of 614 EU m^{-3} (Donham et al. 2000). This threshold level is within the

range of detected aerosolized endotoxin downwind of biosolids loading as determined by this study, suggesting that biosolids workers would be at greatest risk of decreased pulmonary function over a work shift from exposure to aerosolized endotoxin. In addition, biosolids workers come into contact with aerosolized endotoxin on a daily basis as opposed to community exposure, which would typically be no more than 6 days per year as most biosolids applications on a particular field take place over a 3 day period for no more than 2 applications per year (Brooks 2004). It is also important to note that not all endotoxin is highly bioactive, and that most occupational studies can not specifically determine pulmonary effects caused by endotoxin and not as an overall effect of exposure to endotoxin, dust, and other aerosolized compounds. Further studies on these endotoxin exposures need to be conducted to ascertain the level of toxicity associated with endotoxin derived from biosolids and soil associated with the land application of biosolids. This study suggests that community endotoxin exposure from biosolids land application is similar to other agricultural activities not involving biosolids, and that these endotoxin exposures particularly community exposures are within acceptable limits with respect to human health.

ACKNOWLEDGEMENTS

This work was funded by the University of Arizona National Science Foundation Water Quality Center.

Table 1. Aerosol sample types and distances collected throughout the study.

Aerosol Sample Types	Collection Time (min)	Collection Volume (L)	Downwind Distance (m)	# of samples collected
Background (BG)	20	250	0	12
Loading (LD)	10	125	2 – 10	24
Loading (LD)	10	125	40 – 50	15
Slinging (SL)	10	125	10	6
Slinging (SL)	10	125	20 – 25	6
Slinging (SL)	10	125	125 –200	12
Biosolids Pile (PL)	10	125	2	6
Total Operation (TO)	10	125	10 – 200	33
Tractor (TR)	10	125	2	5
Aeration Basin (AB)	10	125	2	6

Table 2. Aerosolized endotoxin concentrations detected downwind of biosolids operations, a wastewater treatment plant aeration basin, and a tractor operation.

			Aerosolized Endotoxin			
Sample Type	# of samples collected	Distane from site (m)	Avg	Median	Minimum	Maximum
				EU m^{-3}		
Controls						
Background	12	NA	2.60	2.49	2.33	3.84
Biosolids Operations						
Loading	39	2-- 50	343.70	91.50	5.60	1807.60
Slinging	24	10 -- 200	33.50	6.30	4.90	142.90
Biosolids Pile	6	2	103.00	85.40	48.90	207.10
Total Operation	33	10 -- 200	133.90	55.60	5.60	623.60
Wastewater Treatment Plant						
Aeration Basin	6	2	627.30	639.00	294.40	891.10
Non Biosolids Field						
Tractor	5	2	469.80	490.90	284.40	659.10

* Avg – Average

* EU m^{-3} – Endotoxin units per m^3

* NA – Non applicable

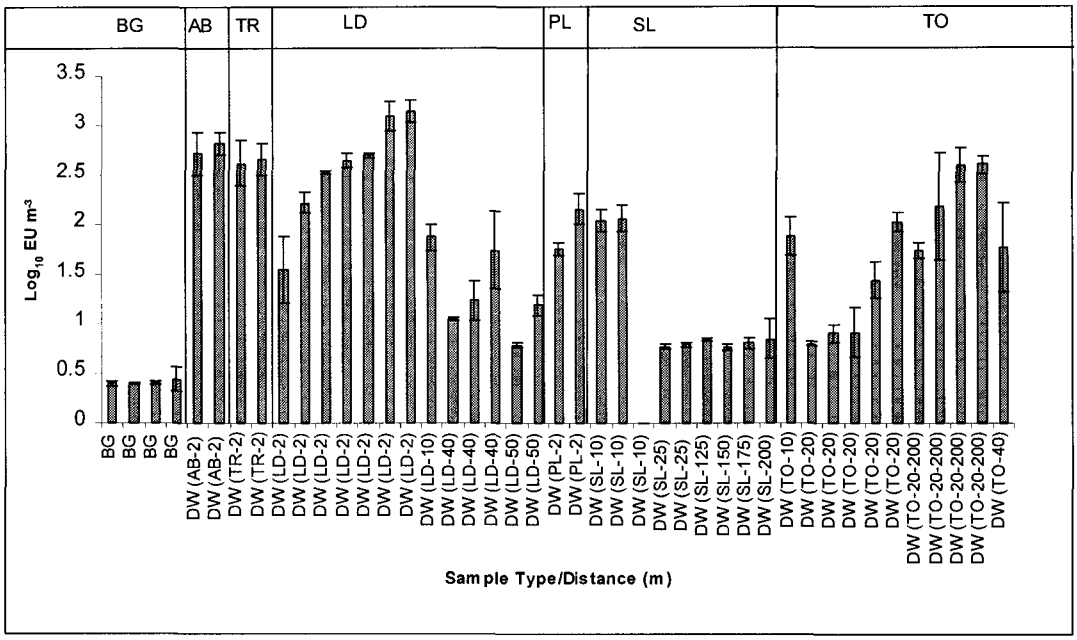

Figure 1. Aerosolized endotoxin concentrations by sample type and distance from source, all bars represent an average of triplicate samples.

* DW – Downwind

*BG – Background, AB – Aeration Basin, LD – Loading, PL – Biosolids Pile, SL – Slinger, TO – Total Operation, TR – Tractor Operation

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