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Proceedings of the Biosolids Exposure Measurement Workshop

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Abstract

Sludges are generated during the processing of wastewater. These sludges are further treated, primarily to reduce the concentration of pathogens, to produce biosolids which are then beneficially used on land. In the United States, the Environmental Protection Agency regulates biosolids through the 1993 40 CFR Part 503 regulations. A 2002 National Academy of Sciences-National Research Council report, "Biosolids Applied to Land: Advancing Standards and Practices," concluded that additional scientific work was needed to reduce uncertainty about human health effects from exposure to biosolids. The final Agency response to this report was published in the Federal Register in 2003. The response included an action plan with 14 projects related to the treatment and disposal of biosolids. One of these projects was to conduct a Biosolids Exposure Measurement Workshop. This workshop was held March 16-17, 2006, at the Andrew W. Breidenbach Environmental Research Center, Cincinnati, OH.

This document is a summary of the workshop. It describes presentations given by 16 experts on issues relevant to measuring human exposure to biosolids contaminants and outlines the topics covered during a panel discussion session. It concludes with a list of research needs that, if met, will enable the environmental community to better evaluate human exposure to biosolids contaminants. In the long-run, the goal of this workshop is to help enable the Agency to better assess the risk associated with the land application of biosolids.

Notices

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Acronyms and Abbreviations

AGI	All glass impinger
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulation
D/T	Dilution/threshold
ELISA	Enzyme-Linked Immunosorbent Assay
FT	Foot
FTIR	Fourier Transform Infrared Spectroscopy
HPC	Heterotrophic plate counts
M	Meter
MIN	Minute
MRA	Microbial risk assessment
NAS	National Academy of Sciences
NCEA	National Center for Environmental Assessment (USEPA)
NDT	National Decontamination Team (USEPA)
NERL	National Exposure Research Laboratory (USEPA)
NHEERL	National Health and Environmental Effects Research Laboratory (USEPA)
NIOSH	National Institute for Occupational Safety and Health
NRC	National Research Council
NRMRL	National Risk Management Research Laboratory (USEPA)
OW	Office of Water (USEPA)
OW-OWM	Office of Water Office of Wastewater Management (USEPA)
OW-OST	Office of Water Office of Science and Technology (USEPA)
PBPK	Physiologically based pharmacokinetic
PCR	Polymerase chain reaction
PI	Principal Investigator
PM2.5	Particulate matter 2.5 µm or smaller
PPM	Parts per million
PPMV	Parts per million by volume
QA	Quality assurance
QAPP	Quality assurance project plan
QC	Quality control
RFP	Request for proposal
SEC	Second
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VOC	Volatile organic compound
VIC	Volatile inorganic compound
WERF	Water Environment Research Foundation
WWTP	Wastewater treatment plant

Introduction

The pathogen and vector attraction reduction of treated sludge is regulated by the U.S. Environmental Protection Agency (USEPA) through the Subpart D requirements of the 40 CFR Part 503 Rule (USEPA, 1993). This regulation was promulgated in 1993 under the authority of the Clean Water Act. A National Academy of Sciences (NAS)-National Research Council (NRC) 2002 report entitled, "Biosolids Applied to Land: Advancing Standards and Practices," noted that there is a limited amount of information on the effect of biosolids land application on individuals living or working near such sites. Although the report states that there is no documented scientific evidence that Part 503 regulations have failed to protect public health, it concluded that additional scientific work is needed to reduce uncertainty about the potential for adverse human health effects from exposure to biosolids. The research needed includes gathering epidemiological data, investigating allegations of health incidents and conducting additional exposure and risk studies in community populations. The report suggested conducting preplanned exposure assessment studies to characterize the effect of biosolid exposure on the community, to identify chemicals and microorganisms in collected field samples, and to measure endotoxin exposure (bacterial cell wall constituents).

These research priorities were further delineated by participants in the USEPA-Water Environment Research Foundation (WERF) Biosolids Research Summit in 2003 (WERF, 2004). Several high-priority research needs were identified, including: (a) the development and deployment of a rapid incident response process to evaluate possible health effects associated with a biosolids land application; (b) the characterization of bioaerosols associated with the land application of biosolids; and (c) the identification of the odor compounds emitted by sludge in the various stages from generation to end use, and the specification of their sensory potencies and mechanisms of generation and release.

The Agency described its action plan for responding to the NRC report in the Federal Register notice entitled "Final Agency Response to the National Research Council Report on Biosolids Applied to Land and the Results of EPA's Review of Existing Sewage Sludge Regulations" (USEPA, 2003). The plan included a list of 14 projects, with the goal being the strengthening of the technical basis for sewage sludge use and disposal regulations. One of these projects was to conduct a workshop that would examine issues relating to the measurement of exposures associated with biosolids.

To address this need, the USEPA hosted a Biosolids Exposure Measurement Workshop on March 16-17, 2006 at the Andrew W. Breidenbach Environmental Research Center in Cincinnati, OH. Seventy-seven people, including researchers from government and academia, regional sludge coordinators, state sludge coordinators, and other stakeholders, participated in the event. This report provides a summary of the workshop, which included presentations by 16 experts, followed by a panel discussion session. The primary focus of the workshop was to identify current measurement tools and to prioritize research on human health effects related to land application of biosolids. The workshop agenda and list of participants are provided in Appendices A and B, respectively. Appendix C is an update on the status of the WERF incident response project. Five technical proceedings papers are found in Appendix D.

Workshop Background and Objectives
Bruce Mintz

Mr. Mintz, an Assistant Laboratory Director for USEPA's National Exposure Research Laboratory (NERL), opened the workshop by discussing its background and objectives. Public concern about this issue is of major importance, particularly when it is unclear whether reported incidences of adverse human health effects are attributable to the land application of biosolids. The NRC Report (2002) found that "there is a lack of exposure and health information on populations exposed

to biosolids," and recommended that "EPA promote and support response investigations, targeted exposure surveillance studies, and a few well-designed epidemiological investigations of exposed populations."

Measuring exposure to biosolids is very complex because of the number of possible contaminants and exposure pathways. The Section 503 Rules identified 14 exposure pathways related to land application of biosolids. This workshop focused primarily on the inhalation and dermal absorption pathways because most of the published complaints describe symptoms that would be associated with these pathways. The objectives of the group panel discussion were to identify protocols for exposure measurement and modeling and to recommend research priorities. The group tried to reach a consensus on priority contaminants, sampling procedures and frequency, methods of analysis, and fate and transport measurement and modeling.

Land Application and Sludge Treatment

James E. Smith

Dr. Smith, with USEPA's National Risk Management Research Laboratory (NRMRL), discussed biosolids and issues associated with land application. Wastewater treatment plants (WWTP) are designed to remove sewage contaminants, resulting in a clean effluent which is discharged into the environment. These contaminants are concentrated in sludge which can be treated to form biosolids that are then beneficially used on land. Approximately 60% of WWTPs treat and land apply biosolids. This accounts for 40% of the total amount of sludge produced in the U.S. Collectively, this provides more than four million dry tons of material per year for land application. [Note: additional data, not available at the time of the workshop, can be found at: www.nebiosolids.org] Biosolids applied to land must meet federal and state disinfection, vector attraction reduction, and chemical concentration requirements (primarily metals) for protecting the public health. The minimal metal concentrations are largely achieved by pretreating industrial wastes. The Section 503 Rule approach for public health protection uses multiple barriers, including disinfection and stabilization (vector attraction control) to reduce pathogens below the detection limit (Class A). If indicator organisms are present in sufficient densities, the biosolids are considered Class B and land use restrictions are then imposed. These restrictions are designed to allow for natural decay of the pathogens.

More information would aid in determining the best way to use biosolids. Some issues to address include studying the aerosols and dusts generated as the material dries, identifying the fate of pathogens, and verifying the effectiveness of Class B disinfection processes in inactivating pathogens. In addition, some biosolid treatment and application processes tend to emit odors and aerosols and so it would be helpful to determine if the public concerns about land applied biosolids are due more to aesthetic issues or to human health effects.

Protocols for the Timely Investigation of Potential Health Incidents Associated with Biosolids Land Application

Alfred P. Dufour

Dr. Dufour, with USEPA's NERL, discussed a December, 2004 WERF workshop on rapid incident response to health complaints from land application of biosolids. Participants in the meeting developed a request for proposal (RFP), based on input about previous cases of reported health effects from the perspective of those affected.

The objectives of the RFP will be addressed in three phases. Phase 1 will involve the development of a protocol for rapid incident response. This will be achieved by: reviewing previous incidents, collecting information on all adverse environmental outcomes, developing a data collection/investigative instrument, consulting with states about conducting investigations, providing investigation guidance, and communicating with health practitioners. In Phase 2, the protocol will be refined after pilot testing at three localities. After the protocol is optimized, recommendations will be made to WERF on how to roll out the protocol nationwide. In Phase 3, a database of biosolids-related investigations will be developed. In addition, guidance will be provided on the communication of the protocol to the public through community dialogs and other mechanisms.

An update on the Rapid Incident Response Project is provided in Appendix C.

Community Perspective of Biosolids Land Application

Maureen Reilly

Ms. Maureen Reilly, with Sludgewatch, outlined her concerns about biosolids policies and practices from her perspective as a member of a community in which biosolids have been land applied. In her view:

- The health, water supplies, quality of life, and property values of rural communities have been compromised by land application of biosolids.
- There is not an effective mechanism for the public to express their concerns about adverse

health effects that they believe to be caused by biosolids applications.

- The WERF rapid incident response project, which will formulate a mechanism to respond to complaints, has been delayed too long.
- The oversight of land application is complicated by the multiple regulations and governmental authorities (federal, state, county, and municipal).
- The 40 CFR Part 503 regulations need rigorous review.
- Since the 40 CFR Part 503 regulations were promulgated they have been made more lenient and are enforced less aggressively.
- There has not been adequate research on pathogens or on the synergistic effects of contaminants that may be in sewage sludge.
- There is the perception that the USEPA is both the regulator and the chief promoter of land application.

Ms. Reilly made several suggestions that would help address her concerns. First, she suggested that a permitting process would allow for better compliance monitoring and should be implemented. Currently, the 40 CFR Part 503 regulations are self-implementing and no written permit is required. Second, Ms. Reilly suggested the risks from land application can be reduced by separating medical and industrial wastes from the general waste stream, and by monitoring application sites for contaminants. Finally, she suggested that complaints should be systematically recorded, responded to, and if required, investigated. In conclusion, in her view, the small amount of money saved by land application is offset by increased illness, hospital costs, and contamination of soil and water.

Current Exposure Measurement Methods and Risk Assessment

Multi-media Sampling at a Biosolids Land Application Test Site

Eric A. Foote

Mr. Foote, with Battelle, reported on an approach utilized for onsite sampling at a biosolids land application site. The research was a collaboration of the USEPA, the US Department of Agriculture (USDA), the North Carolina Department of Agriculture, Battelle, and other supporting groups, agencies, and organizations. This field study, which is one of the projects described in the December 2003 USEPA action plan, was conducted in 2004 on a two-acre plot of grass pasture in Salisbury, North Carolina. This site was selected because it had not received previous biosolids applications. Air and soil sampling were conducted prior to and during the surface application of anaerobically digested and dewatered biosolids, and some sampling continued for several months.

The goals of this research study were to develop a multi-media sampling approach for airborne and soil-bound contaminants resulting from the land application of biosolids, and to optimize this sampling approach for use in future studies. The study measured air emissions and their short-range transport, and soil microbial concentrations at and around the test site, with a focus on selected constituents including particulates (endotoxins), microorganisms (bacteria, fungi, and viruses), and volatile compounds (including malodorants). Prior to application, the biosolids were analyzed for these compounds. Biomass loadings, microbial population dynamics, endocrine disruptor concentrations, and agronomic characteristics also were determined.

The objectives of the bioaerosol and particulate matter sampling plan developed by the USEPA were to characterize the type and concentrations of select biological agents and particulates at several exposure points within 50 meters of the applied biosolids. The analyses used included ones to detect and measure: heterotrophic bacteria, fecal coliforms, *Escherichia coli, Salmonella, Staphylococcus aureus, Enterococcus* sp., *Clostridium perfringens*, total bacterial bioburden, fungus, enteric virus, coliphage (MS2), and particulate size fractions. The sampling process utilized both mobile and stationary bioaerosol sampling units which included SKC BioSamplers® (impingers) and Andersen 6 stage samplers (impactors).

The objectives of the volatile organic compounds (VOC), volatile inorganic compounds (VIC), and odor monitoring measurements were to determine the presence and concentration of select compounds, both upwind and downwind of the application area. A gas-chromatography method was used to measure sulfur and nitrogen compounds. A Fourier Transform Infrared Spectroscopy (FTIR) unit was used to take real-time measurements of VOCs in the field. Flux chambers were used in the field to measure VOCs. Flux samples were taken directly from the ground prior to application, from the biosolids pile, and from the ground one and two days after application. A portion of the flux samples were analyzed by an odor panel; odor measurements were also made in the field with the Nasal Ranger™ Olfactometer.

The objectives of the soil monitoring were to measure: the amount and distribution of biosolids applied to the field; the concentration of various microbial contaminants over time; the concentration of endocrine disruptive chemicals as a function of depth and time; the microbial community as a function of depth and time; and ecotoxicity before and after biosolids application. Measurements were taken to detect the presence of fecal coliforms, some pathogenic bacteria, enteric viruses, coliphage (MS2), and viable helminth ova.

This study required a considerable level of coordination and communication among the research collaborators because of the large study site. Quality assurance/quality control (QA/QC) was complicated and involved different agencies and offices. Data from this project are a landmark for multi-media information gathered from one site and may serve as a baseline for future studies. A report on this study is in preparation.

Pathogen Risk Assessment for Biosolids: Recent Developments

Jeffrey A. Soller

Mr. Soller, with Soller Environmental, discussed a two-phase WERF-funded project to develop a methodology for assessing the risk of pathogen exposures from biosolids. Microbiological contaminants of concern include viruses, bacteria, protozoan parasites, and helminths from a number of potential human exposure pathways. The following exposure pathways were investigated in the study: direct ingestion of biosolids-amended soil, ingestion of groundwater containing microbes leached through soil from land applied biosolids; and exposure to wind transported microbes from land applied biosolids. Potential important issues in determining appropriate microbial risk assessment (MRA) methods include both exposure-specific parameters, such as magnitude and frequency of exposure, and pathogen-specific factors, such as infectious dose, disease, and infectivity. The study results indicate that it is both feasible and reasonable to apply a dynamic population-based MRA method to estimate human health risk from pathogens in land applied biosolids. The framework was completed in 2002 and published in a WERF report (2003) and in Eisenberg et al., 2004. A demonstration of the methodology has recently been completed.

Sludge Fate and Transport

Analyzing Biosolids for Microorganisms to Achieve Regulatory Compliance

Mark C. Meckes

Mr. Meckes, with USEPA's NRMRL, discussed an evaluation of analytical methods for microbial monitoring in biosolids materials. Current federal regulations require monitoring for indicator microorganisms under some conditions for Class B biosolids and under all conditions for Class A biosolids. Standard protocols for quantifying these organisms were specified in the regulations; however, these protocols were not designed for biosolids. Two alternatives for meeting Class A requirements include monitoring for enteroviruses and viable helminth ova. A guidance document, "Control of Pathogens and Vector Attraction in Sewage Sludge" (USEPA, 1992), includes a plaque assay for detection of total culturable viruses and a protocol for enumeration of viable helminth ova in biosolids.

Recently, methods used for analysis of fecal coliforms and *Salmonella* were reviewed and a standard protocol was developed for biosolids applications. These protocols were evaluated by testing various types of biosolids at twelve laboratories throughout the U.S. Two multiple fermentation tube methods were evaluated for fecal coliforms in biosolids, the lauryl tryptose broth (LTB) - EC broth method and the A-1 method. A single method for *Salmonella* was evaluated, the modified semi-solid Rapaport-Vasiliadis procedure. Results showed considerable variation between laboratories depending upon the biosolids evaluated. The fecal coliforms and *Salmonella* methods have been included as USEPA methods under 40 CFR 136. A similar study evaluating methods for the detection and enumeration of viruses has been initiated with the University of Cincinnati.

Fate and Transport Models

Charles P. Gerba

Dr. Gerba, with the University of Arizona, discussed the major factors to be considered in modeling fate and transport of biosolids pathogens. The purpose of developing these models is to provide a better characterization of the exposure component of a risk assessment. Some of the challenges in modeling microbial transport are that: there are many different species of microbes, some microbes may multiply in the environment, and organisms are not evenly distributed in the environment because they are particulate. Furthermore, their survival and transport rates are affected by weather and climate. Factors that need to be included when modeling pathogen survival in soil or water include temperature, soil moisture, and rate of moisture loss. A major factor influencing virus survival in the subsurface is temperature, which can be easily measured and modeled. A more challenging factor is that viral migration rates vary and are influenced by soil type and saturation.

Dermal Exposure

Non-invasive Assessment of Dermal Exposure

Karla D. Thrall

Dr. Thrall, with Pacific Northwest National Laboratory, discussed the use of physiologically based pharmacokinetic (PBPK) modeling to non-invasively measure dermal adsorption. Volatile chemicals in biosolids may be absorbed through the skin from the soil. This compound can then pass into the bloodstream and other tissues. The absorbed compound either remains in the tissue or blood, or returns in the venous blood and is exhaled. With each breath, some of the compound is volatilized and exhaled, and by measuring the exhaled air the amount of chemical absorbed can be predicted. Breath analysis is conducted using a mass spectrometer that takes real-time measurements. The sample collection device is very simple, and has been used on rats, mice, primates, and human volunteers. Real-time breath analysis and PBPK modeling are ideally suited to track the kinetics of dermal exposure. The methodology is sensitive; therefore, human studies can be conducted at low exposure concentrations. Deployment of the technology with PBPK modeling could improve industrial hygiene practices by enabling onsite measurement of human exposure.

Odors and Irritants

Odor, Irritation and Health Symptoms from Biosolids

Pamela H. Dalton

Dr. Dalton, with the Monell Chemical Senses Center, discussed airborne chemicals and odor and their effects on humans. Volatile chemicals associated with bioaerosols and particulates in biosolids may produce ocular/airway irritation as well as emit objectionable odors. These chemicals can therefore cause annoyance, stress, and perception of health risks. Irritation response ranges from mucosal burning to upper respiratory irritation. However, the odor detection threshold is at much lower concentration than the irritation threshold.

Dynamic determinants affecting sensitivity to odor include frequency, intensity, duration, and offensiveness. Women in particular may become more sensitive with intermittent, infrequent exposures. The way people are exposed to odor affects the response to odors. Duration of exposure can dramatically increase the sensory impact and temporal factors play a huge role. One brief exposure does not predict the results from longer exposure.

An important unresolved issue is whether odors associated with land application of biosolids elicit health symptoms through direct action on target organs or from annoyance. Key data gaps in evaluating the effects of biosolids odors include: documenting actual exposure and effects in communities, identifying key odors and irritants in biosolids, and evaluating the response variation due to temporal factors, individual sensitivity, and attitudes and expectations.

Odor Measurements and Impacts from an Experimental Biosolids Land Application Site

Robert H. Forbes, Jr.

Mr. Forbes, with CH2M Hill, discussed a study involving onsite and laboratory analysis of odor samples taken at a North Carolina biosolids land application site before, during, and after application. A certified offsite panel was used for odor analysis of three flux-chamber samples taken on the day before (-1), the day of (0), and the day after (1) land application. Hand-held Nasal Ranger™ olfactometers were used for field assessment of odors. Chemical sensory tubes were used for field measurements of ammonia and a Jerome gold-film analyzer was used for field measurements of hydrogen sulfide. The offsite panel and Nasal Ranger™ test results indicated odor increased on day 0 and 1 when compared to day -1. Odors were not detected more than 200 feet from the application site. Odors began subsiding on day 2, and were not detected by day 4. Ammonia and hydrogen sulfide disperse rapidly in air and were detected only directly above the applied biosolids immediately after application. Most lingering odors appear to be due to organic sulfur compounds such as methyl mercaptan, dimethyl sulfide, and dimethyl disulfide, or nitrogen-based compounds such as trimethyl amine, indole, and skatole. Odors can be reactivated by changes in temperature or rain events.

Particulates

Airborne Particulates: Technologies and Measurement Issues

Patricia D. Millner

Dr. Millner, with USDA, discussed airborne particulates and exposure assessment issues. Airborne particles of concern are those that are breathable and could cause adverse health effects. Whether health effects occur after exposure to particulate matter is dependent on several factors, including: particle size, exposure intensity and duration, the chemical/biological nature of the particles, the length of time that a particle remains in the air, and the presence of pre-existing conditions (e.g., asthma). A number of different air samplers are available such as impingers, cyclones, particle counters, impactors, filters, and dustfall deposit gauges. Each sampler samples at a different rate, ranging from 1.2 L/min to 400 L/min, and has different sample volumes.

Several factors need to be considered when sampling land applied biosolids for aerosols. The application site needs to be sampled before biosolids application with mock conditions and during the application. An upwind reference site also needs to be sampled. Biosolids are applied by a mobile source which typically applies the material in a directional manner; therefore, sampling location and mobility are critical factors in determining exposure. In addition, meteorological data such as wind speed and direction, the analytical method, background levels of particulates, and the effects of sample collection conditions upon the analyte (desiccation or shearing forces) need to be considered in the analytical and sampling plans.

Pathogens and Endotoxins

Biomarkers of Viral Exposure

G. Shay Fout

Dr. Fout, with NERL, discussed a USEPA Office of Research and Development (ORD) study entitled "Salivary Antibody Responses as an Indicator of Waterborne Infections: Pilot Community Study Before and After Installation of UV Treatment." The study is novel both because of its design and because the use of new approaches to determine pathogen exposure. Although this study is aimed at detecting exposure to pathogens in water, it could serve as a model for future studies aimed at determining the health risks associated with the application of biosolids.

This intervention study has been designed to correlate pathogen occurrence in water with exposure and health effects. Specifically, individuals will be surveyed before and after a new drinking water treatment plant with ultraviolet disinfection goes online. Volunteer families will provide health data by completing questionnaires and by providing monthly saliva samples. These samples will be tested for antibodies to *Cryptosporidium*, norovirus, and rotavirus. In addition, water samples will be taken to determine the occurrence of these waterborne pathogens.

In addition to the unique overall design, this study takes advantage of a new approach to detect exposure by testing for the presence of specific biomarkers (antibodies) found in saliva samples. Traditional indices for estimating pathogen exposure in a community include: individual symptom surveys, school illness records, and pharmaceutical sales. Unfortunately, these indices are not effective for estimating the risk from biosolids exposure because of the lack of a proper control population. In addition, these indices are generally not pathogen specific. In contrast, biomarkers for measuring exposure, such as direct pathogen assays and antibody responses in serum or oral fluids, can more directly target individuals that are likely to be exposed as well as control individuals who are not affected. In addition, antibody assays are pathogen specific and can be used to approximate the time of exposure.

The specific saliva-based assay used in this study, which takes advantage of a novel fluorescent bead array LiquiChip™ technology to determine antibody levels, has several advantages. Unlike a serum-based assay for biomarkers, a saliva-based antibody assay is not invasive. This feature allows individuals at a range of ages to participate, enhances participation of the entire community, and provides significantly reduced specimen collection costs. In addition, the LiquiChip™ assay is more sensitive and reproducible than the standard ELISA with lower costs and analysis time.

Community and Occupational Risk from Bioaerosols during Land Application of Biosolids

Ian L. Pepper

Dr. Pepper, with the University of Arizona, discussed two studies evaluating occupational and community risk from bioaerosols during land applications of biosolids. Bioaerosol samples were collected with SKC BioSampler® impingers from land application sites located across the U.S., using different application practices, such as liquid spray and "cake" application. Samples were collected from downwind and background sites. The concentrations of several microbes were measured, including: heterotrophic plate count bacteria (HPC), total coliforms, coliphage, *Clostridium perfringens*, *Escherichia coli*, endotoxin (lipopolysaccharide), enterovirus, norovirus, and hepatitis A virus (HAV). Overall, in 500 samples analyzed, the levels of bacteria and phage were at or below detection limits by culture methods, and only three samples were positive for norovirus by reverse transcription PCR. Calculated risk of infection in the community was determined to be at or below a 1:10,000 risk of annual infection. Endotoxin concentrations observed during land application were similar to those

observed from tractor operations without biosolids, implying that the endotoxins are from the soil.

Occupational risk studies included three experiments to characterize the concentration of aerosolized microorganisms from biosolid applications, the plume created during land application, and the occupational risk of infection from land application of biosolids. More than 300 air samples were collected downwind of biosolids application sites using liquid impingers, and over 100 samples were collected downwind of microbially seeded, land applied water samples. The seeded water served as a model system for tracking the plume. The aerosolization rates from land application of biosolids were calculated to be less than 33 plaque forming units (PFU) of coliphage and 10 colony forming units (CFU) of coliforms per meter traveled, while the water aerosolization rates were much higher. Exposure duration was brief and limited to the time when the biosolids were in the air. Samples also were taken from air and biosolids at 10 land application sites from across the U.S. and analyzed for coliforms, coliphages, and HPC. The application method strongly influenced aerosolization rates, while relative humidity, temperature and wind speed showed limited effects. Occupational risks of infection from land application are greatest during the loading of biosolids. Both studies indicate that occupational or community exposure to viruses and bacterial indicators is low during and after land application of biosolids, at least for the meteorological conditions evaluated.

Improving the Efficiency of an Impinger for Collecting Bacteria during a Biosolids Application Field Study

Edwin F. Barth

Dr. Barth, with USEPA's NRMRL, discussed the use of impingers for bioaerosol sample collection. Measuring individual airborne exposure accurately is challenging because of the difficulty of matching air sampling devices to different respiratory mechanisms, deposition sizes, and personal breathing heights. In addition, the survival of microorganisms is dependent upon their size, the relative humidity, time, temperature, and UV and ozone exposure. Traditional bioaerosol collection methods include filtration, impaction, and impingement. Advantages of impingement include: longer sampling times (up to 8 hours), the ability to split aliquots for multiple analyses, suitability for molecular methods, and sample direction flexibility. Disadvantages of impingement include the delicate, relatively expensive equipment, and the stress-induced effects on the microorganisms during collection. Research conducted in the field and at the NRMRL Bioaerosol Wind Tunnel evaluated the performance of SKC BioSampler® impingers. The results suggest that modifications to the standard practices involving impinger use will improve recoveries. These modifications include covering of the impinger apparatus to block UV radiation, washing of the inlet neck to prevent drying of the accrued material during sampling, and rinsing the inlet neck and jets after collection to liberate organisms captured in the neck.

Endotoxins

Nancy C. Burton

Ms. Burton, with the Center for Disease Control and Prevention's National Institute for Occupational Safety and Health (CDC-NIOSH), discussed endotoxin sampling techniques and analytical methods. Endotoxins are a lipopolysaccharide complex formed in gram negative bacteria that are chemically and thermally stable. Endotoxins can cause powerful inflammatory reactions in humans with symptoms including fever, flu-like symptoms, cough, headache, asthma, and/or respiratory distress. Other associated health effects include organic dust syndrome and respiratory disease such as asthma. Occupational groups with highly documented exposures include farmers, cotton workers, wastewater treatment workers, trash haulers, and poultry and swine handlers. One method for sampling endotoxins uses 0.4 µm pore size endotoxin-free polycarbonate filters in cassettes, and filter/cassette vacuum collectors. The analytical methods used include the Limulus amebocyte kinetic assay, the kinetic chromogenic method, and the gas chromatography-mass spectrometry method.

Occupational Exposure Assessments at WWTPs

Nancy C. Burton

Ms. Burton discussed exposure routes, contaminants, and sampling techniques used for occupational exposure assessments of workers at WWTPs. These assessments are a conservative estimate of community exposure to biosolids since the contaminant loads are thought to be the highest at the WWTP. In addition, these assessments are site specific because the exposure risks are dependent upon the incoming wastes, treatment process, and job tasks. When identifying potential hazards, it is necessary to relate the environmental evaluation to any reported health symptoms. Assessments at WWTPs should include a consideration of chemical, biological, and onsite physical hazards. The chemical and biological contaminants are primarily concentrated in the sludge.

Exposure routes for chemical and biological contaminants are inhalation, ingestion, and dermal adsorption. Chemicals in the waste stream include

gaseous by-products (carbon monoxide, methane, ammonia, and hydrogen sulfide), dewatering agents, and trace metals. Human pathogens that may be found in sludge include bacteria, viruses, protozoa, and helminths. There are no specific occupational exposure criteria to infectious agents in wastewater or sewage. Biological contaminants are identified through a variety of approaches, including cultural techniques, microscopic analysis (spore traps and surface), and polymerase chain reaction (PCR) techniques. Some considerations for field monitoring of biological agents include sample storage, transport, aerosol range, and sampling and analytical methods.

Panel Discussion Session

Bruce Mintz charged the workshop participants to recommend an exposure measurement protocol, and to identify research needs for measuring exposure. In addition, several other topics related to incident response and biosolids practices were discussed. Because of the complexity of biosolids and the multiple exposure pathways, an exposure measurement protocol framework could not be recommended at the present time. However, it was agreed that the protocol should include multiple exposure routes and contaminants, much like the approach used in the North Carolina field study. The following is a summary of the panel discussion.

The development of an exposure measurement framework is hindered by a lack of knowledge about the nature of complaints and health effects associated with biosolids land application. The lack of an incident response protocol prevents health complaints associated with land applications from being systematically recorded by local and state authorities. It also prevents the classification of complaints by symptom (such as respiratory effects, gastroenteritis, skin irritation, odor/nuisance, and headaches) or by exposure pathway (air, soil, or water). Epidemiological studies of the effects of biosolids land application are needed, with better analyses of baseline and post-application exposure and health information. In most situations there are only a few complaints; how well this correlates with the total number of people who may be affected is unknown. It is also unclear if there is a relationship between complaints and such factors as educational level or health insurance coverage. To better evaluate community exposure, complaints and health effects should be mapped around the application site with the prevailing weather conditions indicated. Real time measurements are needed. This information would make it possible to define the relationship between the number and severity of complaints and the distance from application site.

The next theme discussed was biosolids treatment practices. The panel felt many complaints are likely due to the odor of the applied biosolids. The 503 regulations were designed to reduce pathogens and vector attraction but not control odor. The relationship between vector control, odor generation, treatment practices, and digestion level needs refinement. In addition, the effects of various storage and curing practices need to be evaluated when sludges are composted. Many biosolids products do not generate complaints but some products and operations are problematic. As such, treatment practices should be optimized when necessary. Some alternatives to improving treatment would include applying biosolids in remote areas or injecting them into the soil. The panel felt that exposure and complaints could be greatly reduced by changing the recommended best practices for sludge treatment and biosolids application processes so that odors were reduced.

The third theme discussed was the use of biosolids indicators. A biosolids indicator could be used to trace movement of contaminants after application, to study decay rates, and to plot source curves. Caffeine, fibers, steroidal aerosols, specific microbes, fugitive dust or other substances that occur in biosolids are possible targets. A true indicator would need to distinguish biosolids from manure or septage. Since indicators do not provide specific information about the cause of a complaint and do not necessarily correlate with the transport properties of a specific contaminant, they may be only useful as a screening or research tool.

The final themes discussed were exposure measurement, analytical methods, and research priorities. To answer basic exposure questions, the broad and indicator-based exposure measurement approach taken in the North Carolina field study is appropriate. Indicators would not be appropriate for incident response because the sampling and analytic approach needs to be specific to the complaint and site. This means that the compounds or organisms associated with complaints need to be identified. In general, analytical methods may need to be developed, and more real-time monitoring is needed before, during, and after the application of biosolids both onsite and in the surrounding community.

During the panel discussion, several research needs became apparent, especially in the area of pathogen

and odor exposure. The evaluation of exposure to microorganisms associated with biosolids application, particularly aerosolized pathogens, needs further research. The health effects of fungi may need to be evaluated because soil has a higher fungal burden after biosolids application. Adding to the complexity of monitoring is the interpretation of results due to the fact that microorganisms are present without biosolids application; therefore, ambient background levels need to be determined. The sample planning process should carefully consider the number of samples needed so that the amount of exposure can be accurately estimated. Sampling methods, such as impactors and impingers, should be evaluated to define both their recovery rate and effects upon the stress-sensitive microorganisms that are trapped.

Measurement of exposure to odors also has many research gaps, in part because irritation and health effects from chemical aerosols are dependent upon dose, duration of exposure, and concentration. Other undefined volatile chemicals, not just hydrogen sulfide and ammonia, cause odor in biosolids. These compounds are likely to be found at extremely low concentrations, vary among sites, and have different migration rates and detection thresholds than hydrogen sulfide or ammonia. The time of day also affects odor sample results with the odors being strongest overnight. In addition, the health effects caused by tasteless, odorless materials found in biosolids (PM2.5 or chemicals) need to be determined.

Priority Research Needs

1. Characterize the sites, biosolid products, and biosolids application processes that generate complaints, and change either the sludge treatment or biosolids land application processes.
2. Identify the source of complaints and health effects; specifically, the microorganisms or chemicals that cause complaints. If possible correlate field measurements to health effects.
3. Improve data collection and analysis of complaint incidents.
4. Measure microbial exposure levels before and after land application in controlled long-term research projects.
5. Characterize VOC and odor emissions from land application sites, and other agricultural sites.
6. Evaluate and improve bioaerosols exposure methods, including the direct comparison of microbial sample collection systems.
7. Measure real-time endotoxin concentrations at application sites and other agricultural sites.
8. Measure odor at land application sites before, during, and after application. Compare the odor levels to upwind and downwind sites.

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Appendix A
Biosolids Exposure Measurement Workshop Agenda

Andrew W. Breidenbach Environmental Research Center
Cincinnati, OH

Thursday, March 16, 2006
Introduction Session

8:30-9:45 Workshop Background and Objectives
Bruce Mintz, USEPA
Land Application and Sludge Treatment
James E. Smith, USEPA
Protocols for the Timely Investigation of Potential Health Incidents Associated with Biosolids Land Application
Alfred P. Dufour, USEPA
Community Perspective of Land Application
Maureen Reilly, Sludgewatch

9:45-10:00 Break

Current Exposure Measurement Methods and Risk Assessment

10:00-11:00 Multi-Media Sampling at a Biosolids Land Application Test Site
Eric A. Foote, Battelle
11:00-11:30 Pathogen Risk Assessment for Biosolids: Recent Developments
Jeffrey A. Soller, Soller Environmental

11:30-12:45 Lunch

Sludge and Fate and Transport

12:45-1:15 Analyzing Biosolids for Fecal Coliforms and Salmonellae
Mark C. Meckes, USEPA

1:15-1:45 Fate and Transport Models
Charles P. Gerba, University of Arizona

Dermal Exposure

1:45-2:15 Non-invasive Assessment of Dermal Exposure
Karla D. Thrall, Pacific Northwest National Laboratory

Odors and Irritants

2:15-2:45 Odor, Irritation and Health Symptoms from Biosolids
Pamela H. Dalton, Monell Chemical Senses Center

2:45-3:00 Break

3:00-3:30 Odor Measurements and Impacts from an Experimental Biosolids Land Application Site
Robert H. Forbes, Jr., CH2M Hill

Particulates

3:30-4:00 Airborne Particulates: Technologies and Measurement Issues
Patricia D. Millner, USDA

Pathogens and Endotoxins

4:00-4:30 Biomarkers of Viral Exposure
G. Shay Fout, USEPA

Friday, March 17, 2006

8:40-9:10 Community and Occupational Risk from Bioaerosols during Land Application of Biosolids
Ian L. Pepper, University of Arizona

9:10-9:40 Improving the Efficiency of an Impinger for Collecting Bacteria during a Biosolids Application Field Study
Edwin F. Barth, USEPA

9:40-10:10 Endotoxins
Nancy C. Burton, CDC-NIOSH

10:10-10:30 Break

10:30-11:00 Occupational Exposure Assessments at WWTPs
Nancy C. Burton, CDC-NIOSH

11:00-2:00 Panel Discussion Session
(Lunch during session)

Appendix B
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Appendix C
Update on Incident Response Project

Project Signed, Work Begun on Timely Incident Response

A new WERF research effort, Methodology for Implementing a Timely Incident Response Mechanism (03-HHE-5PP), was started under the lead of Principal Investigator Steve Wing with the University of North Carolina. The first phase of the project will develop a protocol to be used in conjunction with established public health investigation procedures and implemented through the existing network of public health organizations. The protocol will be designed to help determine if, among all potential causes, reports of illness in a community could be associated with biosolids land application or possibly other soil amendment practices.

Proper implementation of the protocol by local, state, and federal agencies could provide information about the occurrence of reported symptoms in proximity to biosolids land applications sites and the times and places where reports are more common. Implementation of the protocol could also help provide the basis for conducting more definitive studies of causal links between human exposures from all potential sources and health effects. The current project does not include implementation.

This project was the highest ranked priority at the 2003 Biosolids Research Summit, during which a group of nearly 75 individuals representing agencies, conservation groups, wastewater facilities, academia, and citizens identified their most pressing research needs regarding land application of biosolids. This research responds to a July 2002 report from the National Research Council (NRC) of the National Academy of Sciences regarding potential health risks related biosolids.

Oct. 5, 2006

http://www.werf.org/press/News_Events.cfm

Appendix D
Technical Reports

Appendix D contains five technical reports that expand upon the information presented at the workshop. The titles and authors of the technical papers are:

- Non-Invasive Assessment of Dermal Exposure *by Karla Thrall*
- Odor, Irritation, and Health Symptoms from Biosolids Land Applications *by Pamela Dalton*
- Detection and Measurement of Odor, Ammonia, and Hydrogen Sulfide Emissions as Part of USEPA Biosolids Land Application Research Project *by Robert Forbes*
- Community and Occupational Risk from Bioaerosols during Land Application of Biosolids *by Ian Pepper*
- Lessons Learned Regarding the Use of an Impinger for Collecting Airborne Bacteria during a Biosolids Application Field Study *by Edwin Barth*

The first four papers were submitted by scientists not employed by the USEPA. As such, these papers were not reviewed by the Agency prior to inclusion in these proceedings. The last paper by Edwin Barth, an Agency scientist, was reviewed before publication in accordance with USEPA policy.

Appendix D-1
Non-Invasive Assessment of Dermal Exposure

Karla D. Thrall
Pacific Northwest National Laboratory

Abstract

Realistic estimates of dermal bioavailability following exposures to volatile solvents in the workplace, or through contaminated soil and water, are critical to understanding human health risk. Compared to dermal exposures with neat or aqueous compound, little is understood about the dermal bioavailability of solvents in soil, dust, sludge, or sediment matrices. A method has been developed to determine dermal uptake of solvents under non-steady state conditions using real-time breath analysis. The exhaled breath data is subsequently analyzed using physiologically based pharmacokinetic (PBPK) models to estimate the dermal permeability coefficient (Kp). This approach has been utilized in both experimental animal and controlled human exposures, with studies conducted to compare the impact of exposure matrix, occlusion versus non-occlusion exposure techniques, and species-differences. To date, studies have clearly illustrated that the methodology is sufficiently sensitive to enable the conduct of animal and human dermal studies at low exposure concentrations over small body surface areas, for short periods of time. Further, the portability of the system allows the methodology to be used to conduct occupational and environmental exposure assessments to volatile compounds.

Introduction

Traditional exhaled breath analysis techniques have involved collection of breath samples in Tedlar bags or stainless steel canisters, followed by laboratory analysis – generally by gas chromatography. Disadvantages of these techniques include the possibility that the collection device may alter the integrity of the sample, the time-consuming and costly analysis of the sample, and the delay between sample collection and analysis. To overcome these disadvantages, a breath-inlet device for a mass spectrometer was developed to allow for the continuous real-time analysis of undiluted exhaled air from experimental animals and humans (Thrall and Kenny, 1996; Thrall et al. 2001). The applications of this system in studies ranging from occupational exposure assessment to applied research are described here.

Exhaled Breath Analysis System

The breath monitoring system utilized in all the described studies consisted of an inlet device connecting a human volunteer directly with a mass spectrometer. The subjects' exhaled breath is passed through a heated large-diameter transfer line into a heated glass-mixing chamber (1.3-L volume). A breath sample enters the mixing chamber via a tube that bends off to one side, and exits the chamber via a tube bending in the opposite direction, thus maximizing turbulence and mixing. A mass spectrometer continually withdraws air samples from the center of the mixing chamber at a calibrated rate. Excess exhaled air is vented from the mixing chamber via a large diameter bore-hole exit tube with negligible flow restriction.

For rodent studies, animals are individually placed in small off-gassing chambers. The animals are awake and unrestrained while in the chamber. Hospital grade (grade D) breathing air is supplied to the animal through the lid of the chamber at a calibrated rate of approximately 200 ml/min. The mass spectrometer continually withdraws air samples from the mixing chamber through a port in the chamber lid at the same rate of approximately 200 ml/min. The concentration of compound in the chamber is used to represent exhalation from the animal.

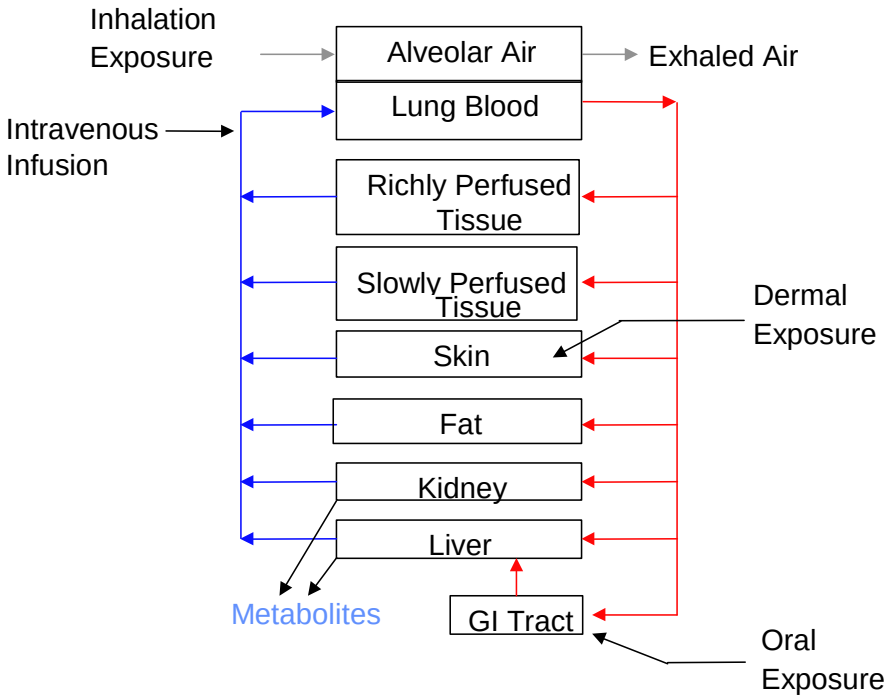

Figure 1: General structure of a PBPK model used to describe pharmacokinetics, tracking uptake from an oral, intravenous injection, inhalation, or dermal exposure, distribution into tissues of concern, metabolism, and elimination of the parent compound and/or metabolites in exhaled breath, blood, urine, and feces

Exhaled breath data can be related to total exposure and internal target tissue dose through the use of physiologically based pharmacokinetic (PBPK) modeling. These physiologically relevant models are powerful tools that can be used to simulate a chemical exposure, regardless of route, and estimate the amount of the compound or its metabolite at a particular internal target. A PBPK model is based on physiology (tissue or organ volumes, blood flow rates, breathing rates, gender-specific physiology, etc.), chemical characteristics (partition coefficients, density, binding characteristics, etc.), and biochemical factors (enzyme-specific metabolic rates, etc.). These factors are either available in the literature, or can be determined in focused in vitro or in vivo studies.

A PBPK model, such as illustrated in Figure 1, describes the body as a series of tissue compartments representing the probable route(s) of exposure, the metabolically active tissues, target organs, and excretion pathways. These models are typically developed and experimentally validated using common laboratory animals, then extrapolated to represent man. A series of differential equations are used to mathematically describe the absorption, tissue distribution, metabolism and elimination of a compound in the body. Once experimentally validated, a PBPK model will facilitate extrapolation across different routes of exposure, from high-to-low doses, and among animal species (Andersen et al. 1993). Thus, by monitoring exhaled breath for a particular compound, the estimated exposure and target tissue dose can be determined.

Application in Dermal Bioavailability Studies

The combination of real-time breath analysis and PBPK modeling provides an opportunity to follow the changing kinetics of the uptake, distribution, and elimination phases of a compound throughout a dermal exposure. The sensitivity of the mass spectrometer for exhaled-breath analysis has been pivotal in enabling studies wherein human volunteers are exposed to low levels of compounds for short periods of time. For example, a method was developed to determine dermal absorption of solvents under non-steady-state conditions by monitoring exhaled air using real-time breath analysis in rats, monkeys, and humans. Dermal patch systems were developed to expose experimental animals and human volunteers to volatile chemicals in soil and water matrices under occluded and non-occluded conditions. The occluded system consisted of a hand-blown glass cell with a needle-hole opening in the top to allow addition of the dosing solution. The needle hole was sealed using silicone following addition of the test

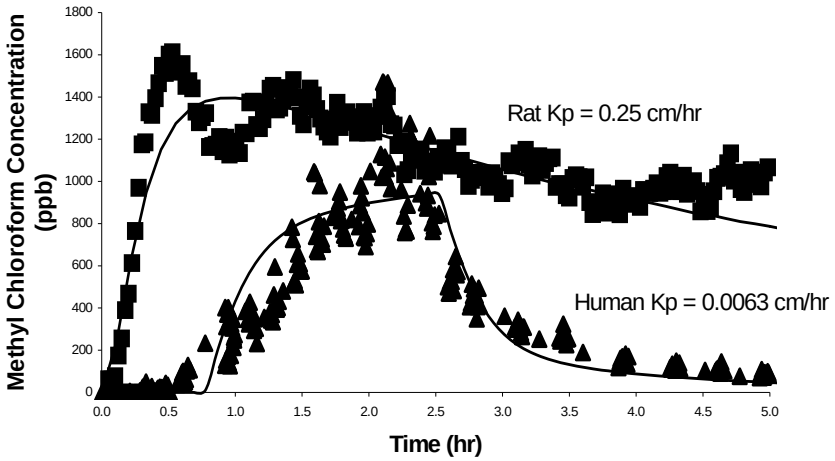

Figure 2. Comparison of PBKB model predictions (lines) and exhaled breath (human) or chamber concentration (rat) following dermal exposure to aqueous methyl chloroform. The human subject was a 29-year old Caucasian man, 95.3-kg body weight, 195.6-cm height, and 22.2-% body fat. Data are averaged after every 1-minute interval (Poet et al. 2000).

material. For non-occluded exposures, glass cells were constructed with an upper chamber separated from the matrix by a semi-permeable frit. The upper chamber was packed with activated charcoal to trap volatilized test materials. Regardless of exposure system, the cells were attached to the forearms of human volunteers (two per arm) or a clipper-shaved area on the lower back of the rat using a cyanoacrylate adhesive.

A number of comparisons of dermal absorption have been conducted, including occlusion versus non-occlusion, soil versus water matrices, and species differences. For example, Figure 2 compares rat and human dermal exposures to aqueous methyl chloroform under occluded conditions. Despite the similarity between the rat and human exposure concentrations (0.1% and 0.12%, respectively) and exposure surface areas (1.7% rat and 3% human), there were clear differences in both the time to peak exhalation and the amount of compound exhaled (area under the curve) between rats and humans. The permeability constant (Kp) calculated from these exhaled-breath data using the PBPK model was roughly 40 times higher in the rat than that calculated for the human (Kp = 0.25 cm/hr versus 0.0063 cm/hr).

Discussion

Exposure assessment is a critical component of industrial hygiene and worker health protection programs. Given today's diverse work environments, new and innovative methodologies for exposure assessment are needed in order to fully understand the potential health risks of the individual worker. Routine analysis of exhaled breath may be ideal for tracking occupational exposures, particularly with the advent of field-deployable standardized methodologies. Exhaled breath analysis offers a number of advantages, including being non-invasive, is applicable to a number of compounds, avoids the handling of potentially infectious biological samples, and can be analyzed easily and quickly using the methodology described here. Furthermore, the system described here goes well beyond traditional industrial hygiene exposure assessment methodologies by employing PBPK models to understand the relationship between exposure and internal, target tissue dose.

The studies described here have focused on the utilization of exhaled breath analysis following controlled dermal exposures to understand the contribution of the dermal pathway on total exposure. These studies have illustrated that the methodology is sufficiently sensitive to successfully measure low-level exposures, or exposures to poorly absorbed compounds in humans. The field portability of the system has the potential to place the exposure assessment methodology in situations where it can be used to conduct occupational and environmental exposure assessments to volatile compounds.

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Appendix D-2
Odor, Irritation, and Health Symptoms from Biosolids Land Applications

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Malodors from biosolids comprise one of the chief complaints that are regularly lodged against land applications, particularly those occurring in close proximity to residential communities. In addition to generating annoyance complaints, however, malodor perception often triggers reports of health symptoms among exposed individuals. Distinguishing between exposures to the volatiles from land applications that elicit ocular or upper respiratory sensory irritation or health symptoms from those that elicit only odor-mediated annoyance is a key component in establishing and enforcing exposure guidelines that are protective of residents in nearby communities. Laboratory studies documenting exposure concentrations and multiple levels of response can help to establish thresholds for annoyance, irritation and health symptoms. Such studies can also help differentiate between the degree to which complaints and symptoms are mediated by psychological rather than physiological factors. However, the successful application of these findings to practice requires a better understanding of the relationship between actual community exposures and response thresholds for each level. This approach necessitates an increased focus on measuring the association between the dynamic profile (i.e. intensity, frequency and duration) for key odorants and irritants in biosolids emissions and the perception and response of exposed community residents.

This paper is divided into two broad sections. The first section addresses the factors which promote adverse responses to the volatile organic compounds associated with biosolids land applications while the second section outlines some key components which should be included in a monitoring plan to evaluate exposure and impact of land applications on humans.

A workshop held at Duke University in 1998 (Schiffman et al., 2001) led to a consensus among attendees that there were three paradigms or mechanisms by which volatile organic compounds (VOCs) could generate health symptoms and adverse responses: (1) The VOCs emitted from land applications could individually or in combination produce ocular and/or upper airway irritation, (2) the VOCs could produce objectionable odors which themselves could elicit annoyance, stress and perception of health risks and (3) the VOCs may simply co-occur with bioaerosols and/or particulates, which themselves are eliciting the symptoms. This paper will focus solely on the first two mechanisms for VOC-induced complaints and examine the evidence for each one in turn.

VOCs, Odor and Sensory Irritation

As odorant concentration increases, the sensory effects experienced typically occur in a predictable sequence. At low concentrations, the presence of an odor can be detected against a clean air background, although its quality may not be apparent. The recognition threshold for an odorant is typically three times the detection threshold. With increasing concentrations, an undesirable odor will often bring about annoyance and/or intolerance. For most volatile organic compounds, direct physical effects from an odorant do not occur until the concentration increases to the point beyond trigeminal or sensory irritant activation. Nevertheless, people often report physical symptoms and irritation at concentrations much lower than the irritant threshold.

Sensations of odor and upper airway irritation are often experienced as a unitary phenomenon, principally because most volatile chemicals have the potential to activate two separate, yet interrelated, sensory pathways in the upper respiratory airways: the olfactory nerve, which gives rise to sensations of odor, and the trigeminal, glossopharyngeal or vagal nerves which give rise to temporary burning, stinging, tingling or painful

sensations in the eyes and upper airways (Doty et al., 2004). Chemical stimulation of the trigeminal nerve (known as *chemesthesis*) often combines with stimulation of the olfactory nerve to produce sensations that form an overall perception of a chemical. For example, low concentrations of ammonia produce a distinct odor; however, higher concentrations may also elicit a mucosal burning or tingling, which is the chemesthetic or irritant component of perception. Most chemicals at sufficiently high concentrations are capable of eliciting upper respiratory tract irritation in addition to odor sensations. Because these two sensory pathways (olfaction and chemesthesis) can be activated by a single chemical stimulus, people often experience and report odor and irritation as a unitary perception. For purposes of evaluating the irritant potential of a chemical, this confusion can invalidate self-report methods, such as symptom questionnaires or even ratings of a chemical's irritancy.

The sensory detectability, irritant potential and quality of land application emissions can be determined in a variety of ways both in the laboratory and the field. For example, in the laboratory, bagged samples of air taken from various onsite and offsite locations can be fed into air-dilution olfactometers and presented to panelists for forced-choice detection of the presence or absence of odor. To measure irritation thresholds that are not confounded by simple odor detection, a method known as nasal or ocular lateralization is used. This technique exploits the fact that the presence of a pure odorant cannot be localized to the left or right nostril or the left or right eye, but a chemical capable of stimulating trigeminal receptors will clearly provide a sensation in the nasal or ocular mucosa which can be localized (Wysocki et al., 1997; Doty et al., 2004).

For evaluation in the field, an instrument known as the Nasal Ranger™ can be utilized to actively dilute ambient air samples in a series of known dilution ratios which are then compared with clean filtered air (McGinley and McGinely, 2004). The dilution ratio at which the individual can reliably discriminate the 'stimulus' air from the clean filtered air is the odor threshold. In both cases, this is reported as the dilutions to threshold ratio for the emission. Although not explicitly designed for this purpose, it is possible that a device like the Nasal Ranger™ could be adapted to do field evaluations of the lateralization potential (which nostril or eye is being stimulated) of real-time emissions. Such an adaptation combines the advantage of onsite testing with the sophistication of the lateralization technique for determining whether emissions reach the concentration necessary to elicit sensory irritation.

Monitoring VOCs and Annoyance

Often the odors associated with land application of biosolids, become significant community issues. Exposed residents may claim that the odors are making them ill. Research has shown that for some period of time following land application, malodors are typically present at concentrations greater than those capable of generating odor perception, but at offsite locations, frequently fall well below those concentrations capable of generating sensory irritation or other acute health effects. The challenge then is to identify the reasons behind community reactions to odors and to understand whether the volatile odor chemicals (i.e. odorants) elicit health symptoms through direct physiological mediation or through psychological or stress mechanisms.

One way to think about the sensory determinants of what makes an odor annoying is the acronym FIDO – the Frequency of an odor, the Intensity at which it occurs, its Duration and its Offensiveness. The first three characteristics can be measured analytically with instruments. However, to understand offensiveness, it is necessary to measure people's reactions. This requires understanding not only the primary sensory attributes of an odor, but non-sensory attributes, or the cognitive and emotional factors that can produce heightened odor awareness and annoyance.

Frequency. Frequency of exposure can by itself alter sensitivity. While it is well acknowledged that continuous exposure to a chemical at a fairly steady concentration leads to a decreased intensity or adaptation, most of the odors from land applications are likely to be intermittent in nature and varying in concentration. In studies that evaluated the impact of intermittent exposure to low-level odors via threshold testing, it was found that females of reproductive age exhibited dramatically increased sensitivity to the odor to which they were exposed, while males, pre-pubescent females and post-menopausal females did not change sensitivity (Diamond et al., 2005; Dalton et al., 2002). In the real world, intermittent exposure to VOCs from land emissions, especially at very low levels, may serve to increase their detectability, even when emissions wane.

Intensity. Measuring the number of dilutions to odorant threshold is important to establish the concentration at which an odorant will become detectable, but provides little information about how increasing concentration will affect stimulus intensity. Although many odorous compounds become detectable at approximately the same physical concentration, increases in physical concentration for one odorant may result in much larger changes in perceived intensity than do the same increases in physical concentration for another odorant. Thus, the shape of the psychophysical function and the

slope are important indicators of how intensity (and perhaps how annoyance) will change with concentration, and are not the same for all odorant chemicals that may be found in land applications.

Duration. The duration of exposure is also critically important when evaluating the irritant potential of a volatile chemical. Increased duration of nasal exposure to ammonia, for example, has been shown to also increase the perceived irritant intensity, due to a phenomenon known as temporal integration (Wise et al, 2005). It is likely that many other irritants exhibit similar patterns of integration, thereby placing important limits on the ability to extrapolate from evaluation of irritants for very brief durations (i.e., seconds) to real-world exposures that may last minutes or hours.

Which Odorous Components to Monitor?

It is also important to recognize that emissions from land applications comprise a complex mixture of many different volatiles, some of which have the potential to contribute to odor or irritant impact more than others. For this reason, the choice of which volatiles to monitor is critical in order to understand the sensory impact of any emissions which migrate offsite. The most typical compounds measured from biosolids emissions are hydrogen sulfide (H_2S) and ammonia; H_2S has a readily detectable and objectionable odor at very low concentrations and is often present at a high volume while ammonia can contribute to the irritation impact of emissions. However, due to the highly heterogeneous nature of biosolids, there are many other malodorous compounds which may be emitted during and after land applications and which may contribute substantially to the odor or irritant impact. Both H_2S and ammonia have fairly high vapor pressure and while they readily diffuse into air and may well comprise the majority of the early sensory impact, they are also likely to dissipate more rapidly than other compounds and thus, prolonged sensory impact may well be the result of other compounds (such as amines, indoles) which volatilize more slowly from the application site.

In the laboratory, characterizing the sensory impact from biosolids via methods such as gas chromatography/ olfactometry (Preti et al., 1993; Bazemore et al., 2000), in which human reports of odor qualities in a complex mixture are linked to the output of a gas chromatograph column separation and the subsequent identification, is important for understanding the sensory potency of any biosolids mixture. In the field, real-time measurements of other compounds with high odor potential should be considered. However, if emissions measurements are confined to sentinel compounds, such as H_2S and ammonia, then continuous monitoring should be augmented by regular and frequent evaluation by human detectors using Nasal Ranger™ technology, to ensure that compounds other than those being monitored are not eliciting malodor perception offsite.

To summarize, the choice of when, where and how frequently to measure and the selection of which key components to measure are important features of any comprehensive monitoring plan for assessing the annoyance impact of biosolids land applications on nearby residents.

Cognitive Factors and Perception of Health Risk

Unfortunately, measuring and finding ways to reduce the sensory impact of any volatile compounds from land applications may not always resolve the complaints from people living or working near the land application site. The perception of even weak or transient malodors can also elicit adverse responses via psychogenically-mediated mechanisms, such as annoyance and stress. Although the presence of an odor is a signal of chemical presence, the potency or hedonic nature of an odor sensation does not correlate with its toxic potential. Because many malodors can be smelled in minute concentrations, simply being able to smell the malodor does not signify that it is present in a harmful concentration.

Research in our laboratory has shown that people's reaction to odor and their beliefs about the effects from odor are influenced by a diverse set of factors, including personality traits, personal experience and information or social cues from the community and media. These factors can increase, or in some cases decrease, a person's sensitivity and awareness of environmental odors. In a series of studies, we have demonstrated how the misperception of the risk from odors actually changes a person's sensory perception of odor levels and their perception of well-being (Dalton et al., 1997; Dalton, 1999).

The results from these studies indicate to us that the reaction that people have to odors is not simply due to their sensory impact but is also shaped by the attitudes and expectations that an individual brings to an odor experience. In no way do we wish to minimize the importance of remediating the sensory impact of emissions as the first step in reducing the level of community annoyance and complaints. However, because even small amounts of odorous molecules can generate odors, reductions of 70-80 percent in odor concentration can still result in complaints if neighbors can detect even weak and transient odors and are concerned about the health impact of the emissions. Thus, working with communities and neighbors to provide them with information and to help them understand the nature of the odors, what they represent

and their known effects can be a powerful tool to modify the cognitive factors that often guide and influence community reaction to odor emissions.

Conclusions

Monitoring programs for evaluating the effect of biosolids land applications on human health should focus on closing the data gaps in the following three areas. First, simultaneous documentation of actual exposure concentrations and effects at the observer level in residential communities is a priority. At the present time, much is known about the emissions at the land application site and at the fence line, but few studies have undertaken comprehensive monitoring at the community level. Second, any such program should strive to identify the appropriate odorants and potential irritants for monitoring, which may well be more than just H_2S and ammonia. Finally, response variation within and across communities should be evaluated in light of the composition of the biosolids, the temporal factors (duration and frequency) of exposure and the attitudes and expectations of the exposed community. These factors, taken together, can do much to identify and illuminate the actual and perceived sensory and health concerns from biosolids land applications.

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Appendix D-3
Detection and Measurement of Odor, Ammonia, and Hydrogen Sulfide Emissions as part of USEPA Biosolids Land Application Research Project

Robert H. Forbes, Jr., P.E.
CH2M HILL, Inc.

Abstract

CH2M HILL participated in a research project entitled "Multimedia Sampling at Biosolids Land Application Sites" as a consultant to Battelle Memorial Institute in the fall of 2004. The overall goal of the research was to advance the science of air, soil, and water quality measurements associated with land application of biosolids to agricultural sites. CH2M HILL's role was to detect and characterize odors from the site, and to measure the concentrations of two volatile gases commonly associated with biosolids land application, ammonia and hydrogen sulfide.

Background odor levels prior to biosolids application (Day -1) were measured as "trace" by the onsite field receptors, while the more sensitive, offsite odor panel measured the odor levels at 60-90 dilutions-to-threshold (D/T). This is indicative of the sensitivity differences between onsite and offsite odor measurements. Odors from ground-level flux chambers onsite increased substantially after biosolids application (Day 0) and the following day (Day 1) as measured by the offsite odor panel. Odors measured in the field increased on Day 0 after application as expected, but did not increase significantly above Day 0 levels on Day 1. Odors measured by the field crew began subsiding on Day 2 and returned to background levels at all sampling locations by Day 4.

Both ammonia and hydrogen sulfide were detected at fairly significant concentrations (15 ppmv and 0.17 ppmv, respectively) from the flux chamber exhaust soon after biosolids application on Day 0, but their concentrations at five feet above ground level (where most field measurements were taken) were much lower. Their concentrations had subsided further at all locations by Day 1 and Day 2, returning to background levels by Day 4.

It was concluded that most odors associated with biosolids land application are not from ammonia and hydrogen sulfide, but more likely are from reduced organic sulfur compounds such as methyl mercaptan and dimethyl sulfide, along with nitrogen-based organic compounds such as trimethyl amine, indole, and skatole. Chemical measurements made by others during the field study support these conclusions.

Introduction

CH2M HILL participated in a research project entitled "Multimedia Sampling at Biosolids Land Application Sites" as a consultant to Battelle Memorial Institute in the fall of 2004. The overall goal of the research was to advance the science of air, soil, and water quality measurements associated with land application of biosolids to agricultural sites. CH2M HILL's role was to detect and characterize odors from the site, and to measure the concentrations of two volatile gases commonly associated biosolids land application, ammonia and hydrogen sulfide. Field work began on the day before the biosolids application event (Sept. 29, 2004 = Day -1) and continued through the day of application (Sept. 30, 2004 = Day 0), followed by Days 1, 2, and 4.

Materials and Methods

Odor measurements onsite were made on all five field-sampling days using three hand-held olfactometers called Nasal Rangers™, and the field odor levels were compared with odor measurements made by an offsite odor panel on three of the sampling days (Day -1, Day 0, and Day 1). Field measurements of ammonia were performed using chemical sensory tubes manufactured by Drager™ coupled with a calibrated hand-held

vacuum pump, also by Drager™. Field measurements of hydrogen sulfide were made using a Jerome™ type gold-film analyzer as manufactured by Arizona Instruments, with an internal sample pump and a detection limit of 0.001 parts per million by volume (ppmv).

Three air samples were taken from onsite flux chambers installed by other researchers (McConnell, 2005), pumped into Tedlar air-sample bags, and shipped overnight to St. Croix Sensory Laboratories in St. Croix, MN, where a certified odor panel was used to conduct offsite odor analyses according to a standard and accepted procedure for measuring the intensity of odors. In this procedure, each odor panelist tries to distinguish a diluted odor sample from two other samples that are odor-free blanks. The sample air is mixed with pure, odor-free air at pre-set dilutions. The sample's dilution level at which an odor is barely detected by the panelist is called the "detection threshold" (DT) of that odor sample. The intensity of the odor sample itself is measured in terms of the number of dilutions that were combined in the sample at which the DT was measured, in units expressed as dilutions-to-threshold (D/T). A seven person panel is used in certified, offsite odor testing, and the measurements by different panelists are averaged to arrive at the reported odor DT in odor units of D/T.

Onsite odor measurements taken with the Nasal Ranger™ olfactometer are intended to mimic measurements made by an offsite odor panel, with results of both methods reported in similar odor units (D/T). The offsite odor-panel measurements are usually much more sensitive than onsite measurements, but the relative differences among odor samples should show similar trends with either onsite or offsite odor measurements.

Results

Results of daily odor measurements were compared and trended. Background levels prior to biosolids application (Day -1) were measured as "trace" by the onsite field receptors, while the more sensitive, offsite odor panel measured the odor levels at 60-90 dilutions-to-threshold (D/T). This is indicative of the sensitivity differences between onsite and offsite odor measurements.

Odors from ground-level flux chambers increased substantially after biosolids application (Day 0) and the following day (Day 1) as measured by the offsite odor panel. On the flux-chamber sample that was taken on Day 0 immediately after the land-application event, the offsite odor panel measured odor intensities in the range of 500 to 1,000 dilutions-to-threshold (D/T). The offsite odor panel reported even higher odor levels from flux-chamber samples taken on Day 1 approximately 24 hours after land application, with their results ranging from 2500 to 6100 D/T.

Offsite odor analyses by a certified odor panel are quite expensive, however, so the three flux-chamber samples taken on Day -1, Day 0, and Day 1 were the only samples to be shipped to St. Croix Sensory Laboratories and analyzed there.

The onsite odor panel measured odor levels exceeding 30 D/T from the flux chamber exhaust sample on Day 0 (about an odor of magnitude less than the 500 D/T odor measurements by the offsite odor panel). The onsite odor panel also measured odor levels in the range of 15-30 D/T from ambient air onsite and immediately downwind. At locations immediately upwind of the site on Day 0, the onsite odor panelists measured odors in the range of 2-7 D/T.

On the day after biosolids application (Day 1) the onsite odor panelists once again measured odors from the flux chamber exceeding 30 D/T, while the offsite panel reported even higher odor levels. These results indicate that there may be some acclimation and decreased sensitivities to odor levels by onsite odor panels, as compared with the more sensitive offsite odor panels. Ambient odor levels measured by the onsite field panel were in the range of 15 D/T onsite and immediately downwind on Day 1, while the onsite panelists could not detect any odors above background upwind of the site on Day 1.

Ambient odor levels as measured by the onsite panel began subsiding on Day 2 to a level of 15 D/T from the flux chamber exhaust. Odors were barely detected at locations downwind of the site on Day 2, and were undetected at all locations upwind of the site. Odor levels as measured by the onsite panelists had returned to background concentrations at all sampling locations by Day 4.

Ammonia was not detected in any of the background measurements taken on Day -1. Hydrogen sulfide was detected in the Day -1 background measurements at concentrations of 0.002 to 0.005 ppmv. The hydrogen sulfide measurements are at least partially attributed to the exhaust gases of the biosolids-applicator and sampling vehicles, which were running without biosolids on the day prior to biosolids application (Day -1).

Both ammonia and hydrogen sulfide were detected at fairly significant concentrations (15 ppmv and 0.17 ppmv, respectively) from the flux chamber exhaust samples soon after biosolids application on Day 0. The concentrations of ammonia and hydrogen sulfide at five feet above ground level (where most field

measurements were taken) were much lower, however. Their concentrations had subsided further at all locations by Day 1 and Day 2, and concentrations of both ammonia and hydrogen sulfide had returned to background levels by Day 4.

At least one other researcher at the experimental site detected ammonia using the open-path integrated, optical remote sensing techniques (Harris, 2005), which utilizes absorption infrared spectroscopy. In that research, an ammonia plume was measured during and immediately after biosolids application, but it dropped rapidly to non-detect levels within three hours after application. The results of those ammonia measurements were in the same ranges as results obtained in this study using Drager™ chemical sensory tubes.

Conclusions

It was concluded that ammonia is a source of odors only during and immediately after biosolids application, to receptors that are either onsite or in close proximity to the application site. Ammonia is generally not detected at any appreciable distance from the site because it has a high detection threshold and it disperses very rapidly upon volatilization.

Hydrogen sulfide may be detected in background samples due to its presence in vehicle exhaust, and it also has sources in livestock operations (chickens, cattle and hogs, for example) that are often found in rural areas. Generally, the increase in hydrogen sulfide over background levels due to biosolids application is very slight, and hydrogen sulfide is not generally a significant contributor to odors from most biosolids application activities.

It was concluded, based on the results of this study, that most odors associated with biosolids land application are not from ammonia and hydrogen sulfide, but more likely are from reduced organic sulfur compounds such as methyl mercaptan and dimethyl sulfide, along with nitrogen-based organic compounds such as trimethyl amine, indole, and skatole. Chemical measurements made by others during the field study (McConnell, 2005) tend to support these conclusions.

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Appendix D-4
Community and Occupational Risk from Bioaerosols during Land Application of Biosolids

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During land application of Class B biosolids there is the potential for aerosolization of human pathogenic microorganisms that could adversely impact human health and welfare. Recently the University of Arizona conducted a major study evaluating the community and occupational risk from bioaerosols during land application of biosolids. In addition, the incidence of aerosolized endotoxin was also evaluated. For community risk, aerosol samples were collected for two years from land application sites located at various locations throughout the U.S., representing 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, including: 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.

Community risk assessments were conducted using an empirically derived transport model. (Brooks et al., 2005). The assumptions used in the risk assessment were:

- 10^5 phage/g biosolid
- 0.1 virus/g biosolid (assume Coxsackie)
- virus:phage = 1:1000000
- using the seeded water model for phage and adjusting for biosolid phage concentration, the estimated phage number at 100 ft is 7.16×10^{-1} phage/m^3
- therefore estimated virus concentration from land application of biosolids at 100 ft = 7.16×10^{-7}/m^3
- assume 0.1 coxsackie virus/g biosolid
- distance from site = 100 ft
- exposure = 1 hr
- dose = 5.94 E-07 virus
- using one-hit exponential model ($P = 1\text{-}e^{rN}$)

Risk = 1.50 E-08
Similar Risk for 8-hr exposure = 1.2 × E-07

For a more conservative approach

- assume 100 viruses/g biosolid
- estimated virus concentration from land application of biosolids at 100 ft = 7.16×10^{-4}/m^3
- assume 100 virus/g biosolid

One-hour Risk = 1.5 E-05
8-hour Risk = 1.2 E-04

Figure 1 shows risks determined assuming different viral concentrations within biosolids at different distances from the land application site. Exposure times are also varied.

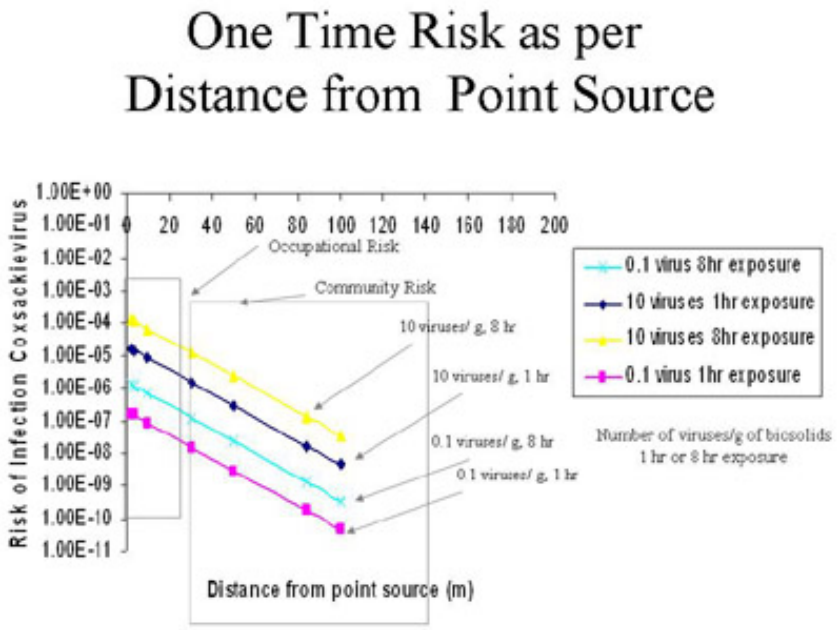

Figure 1. Risk assessments generated at set distances from the land application site assuming different viral inputs and exposures times

For occupational risk, three experiments were conducted to characterize the concentration of microorganisms in biosolids, the plume of aerosols created during land application of biosolids and the occupational risk of infection due to pathogens aerosolized during land application of biosolids in the United States. In all, more than 300 air samples were collected immediately downwind of biosolids applications throughout the United States using liquid impingers, and more than 100 air-samplers were collected downwind of microbially seeded, land applied water, which served as a conservative model system of aerosol generation. The novel model system made it possible to calculate the flux of microorganisms through a virtual plane defined by air samplers in vertical and horizontal arrays, located immediately downwind of a passing spray applicator. The rate of aerosolization during land application of biosolids near Tucson, Arizona, was calculated to be less than 33 plaque forming units (PFU) of coliphage and 10 colony forming units (CFU) of coliform bacteria per meter traveled by the spray applicator (Tanner et al., 2005). Rates of aerosolization from the model system were shown to be much greater. Exposure duration was shown to be brief and limited to the time when biosolids were actually in the air. To assess the risk to occupational health from bioaerosols generated during land application of biosolids, coliform bacteria, coliphages, and heterotrophic plate count (HPC) bacteria were enumerated from air and biosolids at 10 land application sites throughout the nation. The method of land application strongly influenced aerosolization, while relative humidity, temperature and wind speed showed limited correlation to concentrations of fecal indicator microorganisms in air. Occupational risks of infection and illness from aerosolized *Salmonella* and enteroviruses were calculated for a variety of land application scenarios.

The assumptions used in the risk assessment were:

- Worker on land application site 8 hours/work day
- 251 work days/year
- Tractor cab air filter 50% efficient in removing aerosolized microorganisms
- Operator downwind of biosolids 50% of the time
- No inactivation of aerosolized microbe
- Concentration of pathogens and indicators in air will be a reflection of pathogens and indicators in biosolids
- Therefore concentrations of coliforms in air can be used to estimate concentration of *Salmonella* in air, and
- Concentration of phage in air can be used to estimate human virus concentrations in air
- Breathing rate = 10 m^3 /8 hours (light activity)
- 100 to 1000- *Salmonella* and 0.1 to 10 human virus/g of biosolids
- 10% of *Salmonella* that are inhaled are subsequently ingested (no dose response available for inhalation of *Salmonella*)
- Human virus is Coxsackievirus A21 (dose response for inhalation available)

The probability of infection from ingestion of pathogenic bacteria was calculated using the Beta-Poisson Distribution model.

Here $P_{day} = 1 - [1 + (d / N_{50}) (2^{1/\alpha} - 1)]^{-\alpha}$

Where:

P_{day} is the probability of infection per workday

d is the number of pathogens ingested per day (10% of pathogens inhaled)

N_{50} is the dose at which half of subjects are infected with a particular pathogen

α is a parameter which describes the distribution of infection

The probability of infection from the inhalation of virus was calculated using the Single-hit Exponential Distribution Model

Here, $P_{day} = 1\text{-}e^{-rd}$

Where,

r is a parameter defining the probability of a single organism initiating infection

d is the number of pathogens inhaled per day

The annual risk of infection was calculated from the daily risk of infection, assuming 251 days per year of occupational exposure, using the following formula:

Here: $P_{year} = 1 - (1 - P_{day})^{251}$

Calculated occupational risks are shown in Tables 1 and 2 assuming different viral and bacterial concentrations within biosolids.

Summary

- Overall risk of infection from bioaerosols resulting from land application is low.
- Duration of exposure during land application is very discrete.
- Occupational risk is greater than community risk due to enhanced exposure, but still low.
- Community risk is insignificant.
- The greatest risk to occupational workers occurs during loading of biosolids.
- For community and occupational risk, there is less risk due to bacteria than virus.
- Application method influences aerosolization rates.
- Environmental factors do not influence aerosolization rates

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Table 1: Occupational risk: annual risk of infection due to inhalation of coxsackievirus A-21

	Enterovirus/g biosolids		
	10	1	0.1
Loading	1.9×10^{-1}	2.1×10^{-2}	2.1×10^{-3}
Land Application	7.5×10^{-2}	7.8×10^{-3}	7.8×10^{-4}

Table 2: Occupational risk: annual risk of infection from non-typhi *Salmonella* assuming 10% of inhaled *Salmonella* are ingested

	Salmonella/ g biosolids	
	1000	100
Loading	1.3×10^{-3}	1.3×10^{-4}
Spreader	9.8×10^{-5}	9.8×10^{-6}

Appendix D-5
Lessons Learned Regarding the Use of an Impinger for Collecting Airborne Bacteria during a Biosolids Application Field Study

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Abstract

The National Risk Management Research Laboratory (NRMRL) of the USEPA performed laboratory wind tunnel studies as well as large field studies to evaluate specific bioaerosol components (bacteria, fungi, endotoxin, β-d glucan) that may be associated with various practices for managing semi-solids such as biosolids or sediments (contaminated by combined sewer overflows). The wind tunnel studies indicated limitations involving the impingement collection method. Modifications to common practices for impingement collection procedures, gained from the wind tunnel studies, were implemented into the design of the bioaerosol sampling collection plan for a large field study involving the land application of biosolids to increase the likelihood that stress-sensitive bacteria (Gram-negative) could be collected and detected. These modified procedures included: covering the impinger with aluminum foil to block ultra-violet radiation transmission during collection; periodic washing of the inlet neck during collection to prevent drying of the biological mass that may collect on the inlet neck during collection; and a final rinse step of the inlet neck and jet nozzles after collection.

Introduction

Various sampling methods are available to collect airborne bacteria. Interpreting data from any of these methods for use in a human exposure assessment is difficult since the flow rate and mechanisms for particle collection of a biological sampling device may be different from the physiology and particle deposition of the human pulmonary system. Furthermore, some of these sampling devices are relatively large, making it impractical to collect air samples within the personal breathing zone of an individual.

The survival of bacteria in air depends upon physical and chemical factors of the particle such as the particle size and membrane components. Environmental factors also play a role in survival in air such as relative humidity, evaporation rate, temperature, transport time, and ultra-violet radiation. In addition, an “open air factor” also reduces viability (Cox et al., 1973). In contradiction, bacteria have been known to travel long distances, across oceans, if ultra-violet radiation is blocked by particulate matter (Griffin et al., 2002).

Traditional bioaerosol sampling methods include filtration, impingement, and impaction. The advantages of the impingement method for collecting viable microorganisms may include the possibility of longer sampling times (depending upon the collection fluid) to more accurately assess the personal exposure to bioaerosols when a person spends a significant period of time in a specific environment (Lin et al., 1999), less susceptibility to collection medium overload, and an inlet parallel to the air stream. In addition, the collection of the bacteria into a fluid allows the flexibility to split the collection fluid into several aliquots for various types of analysis other than bacteria including endotoxin (Duchaine et al., 2001), viral (Agranovski et al., 2004), and molecular methods (Angenent et al., 2005). Disadvantages of this collection method include delicate sampler device handling procedures, and more difficulty in setting-up and utilizing in the field relative to other bioaerosol sampling equipment devices.

Bacterial enumeration techniques included filtration/ plating of the impinger fluid or a spread plate method used in other bioaerosol impinger studies (Lin et al., 1999). Even with high collection efficiencies of airborne

bacteria, assays for individual microorganisms may result in low recovery. Microbial ecology studies have shown the culturability of microorganisms is low compared to actual counts in many environmental settings (Fabbian et al., 2004). For example, the recovery efficiency for *S. aureus* seeded in a biosolids sample was 8.7% (Rusin et al., 2003). In another bioaerosol study, less than 10% of the aerosolized bacteria were capable of forming visible colonies with culture techniques (Heidelberg et al., 1997).

Materials and Methods

Laboratory Wind Tunnel Studies

The evaluation of the impingement collection procedure consisted of several laboratory trials that were completed prior to a large field trial involving biosolids application. For the laboratory trials, a 0.3 m x 0.3 m x 3.6 m (1.0 ft x 1.0 ft x 12.0 ft) wind tunnel was used to evaluate various bioaerosol sampling methods. Three BioSampler® impingers (SKC Inc., Eighty Four, PA) were located 0.9 m (3.0 ft) downwind of the source area in the wind tunnel. The source area contained various solution concentrations of a stress-sensitive organism, *E. coli,* in a 1000 ml beaker. The bacteria were aerosolized from the beaker by bubble aeration and mechanical mixing. The velocity of the filtered air in the wind tunnel approximated 0.75 m (2.5 ft)/ sec.

Three sets of trials (initial, continuation, threshold) using *E. coli* (ATCC 25922), a fecal coliform bacterium of interest for biologically contaminated sediment, were performed to develop an efficient collection procedure for the impingers for collecting stress-sensitive bacteria in the field.

Two types of impingers were considered for the studies. The design and specifications of the BioSampler® impinger include an inlet neck, collection body, and collection vial. This more recent design is similar to the All-Glass Impinger (AGI-30), which has been available for several years. A major difference between these two impinger types is that particles impinge into the collection fluid at 90 degrees with the AGI-30, while the combination of impaction and centrifugal motion may result in less re-aerosolization of particles from the BioSampler® (Lin et al., 2000). Other studies with the BioSampler® have shown nearly 100% collection efficiency for a wide particle (non-biological) range, decreasing to approximately 90% for 0.5 µm particles and to 70% for 0.3 µm particles (SKC, 2004; Lin et al., 2000). The collection body has a critical orifice in each of the three jet nozzles that result in a flow rate of approximately 12.5 Lpm through the nozzle jets (at sonic flow) at a pressure drop of 0.5 atmospheres (15 in Hg) or more across the sampler (SKC, 2004). From this literature, a decision was made to only use the BioSampler® impinger design in both the wind tunnel and field studies.

The curved inlet neck of a similar impinger design, the AGI-30, collects particles larger than 8 µm that can be recovered with a wash rinse (ACGIH, 1995). Non-isokinetic collection conditions involving the AGI-30 sampler theoretically result in decreased collection efficiency of particles larger than 1.0 µm (Grinshpun et al., 1994). A final rinse procedure, which consisted of five separate 1.0 ml rinses of a phosphate buffered solution (PBS) after collection was used to wash down any organisms that may have aggregated and been in contact with the inlet neck or nozzles during collection.

Field Study

To prevent dry-out of organisms that might be collected in the inlet neck, as well as replace impinger fluid that may be lost to evaporation during outdoor collection activities, periodic rinses of distilled/de-ionized water were introduced into the sampler inlet neck during field collection activities to increase the likelihood that stress-sensitive organisms could be collected. In addition, the impingers were covered with aluminum foil to reduce the chance of any collected bacteria being exposed to ultra-violet light radiation during the collection and sample handling period, which might have a detrimental effect on bacteria.

For the field study, a sampling station layout for the 100 m diameter circular study area (Figure 1) was designed for flexibility in sampler orientation. Multiple sampling zones containing multiple sampling stations were spread apart to increase the size of the collection area of the sampling field, as opposed to clustering the samplers within a narrow region of the field. The sampling station layout included three upwind stations (center station located 16 m upwind from the top edge of the application area), three stations in the first downwind zone (center station located 16 m downwind of the bottom of the application area), and three stations in the second downwind zone (center station located 50 m from the first downwind zone center station). The stations in the upwind zone and first downwind zone were 75 m apart within each zone. The stations within the second downwind zone were approximately 90 m apart. This station layout would allow physical movement of each station (following an arc pattern) because the samplers were secured upon pull carts so that they could be re-positioned with varying wind patterns.

The operational schedule involved periodic operation of the samplers for the period of time when the spreader passed through the application area, with an allowance time for reasonable particulate transport (two minutes) to

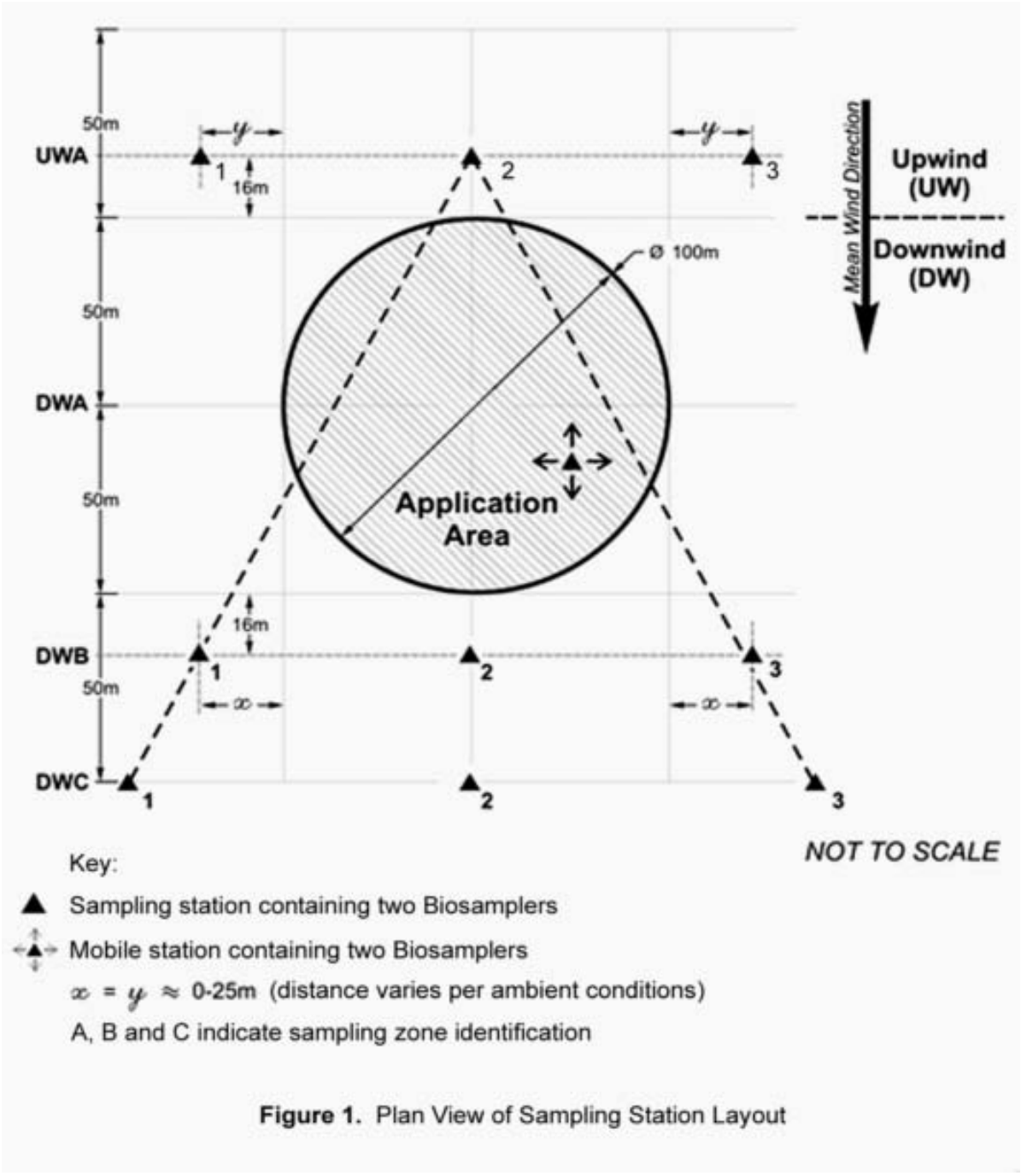

Figure 1. Plan View of Sampling Station Layout

downwind stations, as opposed to continuous operation of the samplers. During the time the samplers were off, distilled water was introduced into the inlet neck. This intermittent operation and rinsing were intended to reduce evaporative loss of impinger fluid associated with outdoor sampling activities.

Results and Discussion

Laboratory Wind Tunnel Studies
Initial Trial Set (trials 1-4)
The initial two trials indicated that the initial sampling procedure using white mineral oil as a collection fluid in the BioSamplers®, for a long time period (120 minutes),

was not effective for capturing the stress-sensitive bacteria (*E. coli*) introduced into the tunnel, as no growth was detected. Various reasons for not being able to culture stress sensitive organisms from the impinger were considered. One reason may be that "clumping" of the bacterial cells may be too large for the bend in the impinger, collecting in the inlet bend, possibly by charge attraction, and remain sensitive to air drying (if collected in the sampler inlet). Another reason could be that the cell viability is reduced due to the agitation in the impinger being too severe. Another reason is the cell viability may be reduced during subsequent filtration, prior to plating. A third trial employed a final inlet and jet wash after collection, and (PBS) instead of oil as the collection medium in an attempt to recover any bacteria. However, to limit air drying, the trial was only performed for 10 minutes, raising the detection limit for this trial, which did not result in detection. A fourth trial using *Bacillus* spores indicated that the impingers could collect less stress-sensitive organisms over a thirty minute collection period.

Continuation Trials (trials 5-7)
The continuation trials ultimately indicated that the impinger operational practice of using PBS, operated for 30 minutes, employing a final inlet/nozzle wash was satisfactory for collecting and culturing stress-sensitive organisms, depending upon the analytical method used for culturing the bacteria.

Threshold Trials (trials 8-11)
The last set of trials (trials 8, 9, 10 and 11) were used to determine if a concentration threshold (initial culture) was necessary to detect organisms in this experimental set-up. These trials indicated that the culturable counts from the impingers were dependent upon the initial solution concentration counts, once above a threshold (greater than 10^5 CFU/ml) for this particular wind tunnel design.

Field Study
The impingers were operated during both a control trial and biosolids application trial following the sampling and analytical plan. The re-positioning of the sampling stations due to changing wind patterns was difficult to implement because it was difficult to communicate and coordinate sampling station movement among all sampling personnel. The intermittent operation of the samplers and associated rinsing steps was able to be implemented during the biosolids application period, using pre-sterilized packets of water. Detectable bacteria concentrations were observed for the impingers at each of the sampling stations. Complete statistical review of this data will be performed in the future.

Conclusions

Impingers may offer some advantages for collecting and analyzing a large variety of bioaerosol components as compared to other bioaerosol collection methods which typically collect only a single biological agent. Operational strategies are available to potentially increase the likelihood that stress-sensitive bacteria, such as Gram-negative bacteria, can be collected and cultured from air samples. A collection procedure involving periodic operation and rinsing of the foil covered impingers, followed by a final rinse step, resulted in the collection of viable bacteria during a field application study.

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