CONTEMPORARY PERSPECTIVES ON

INFECTIOUS DISEASE AGENTS IN SEWAGE SLUDGE AND MANURE

EDITED BY: JAMES E. SMITH JR. • PATRICIA D. MILLNER
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Based on the proceedings of the Workshop On Emerging Infectious Disease Agents And Issues Associated With Sewage Sludge, Animal Manures, And Other Organic By-Products, June 4 - 6, 2001, Cincinnati, Ohio, June 2001. Peer Reviewed and Updated, 2003-2004.

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The need for and concept of a joint workshop on the state-of-the-knowledge on infectious agents and disinfection technologies for sewage sludge and manure developed from a series of discussions in 1999 and 2000 among Dr. J. E. Smith, Jr. of the U.S. EPA's Pathogen Equivalency Committee, Dr. Patricia Millner, Environmental Microbial Safety Laboratory, USDA, Agricultural Research Service, and Dr. John Walker of the U.S. EPA's Office of Water. Funding for the workshop was provided by the U.S. EPA's National Risk Management Research Laboratory and the USDA's Agricultural Research Service.

Executive Summary

The United States Environmental Protection Agency (USEPA) and the United States Department of Agriculture (USDA) convened a three-day Workshop On Emerging Infectious Disease Agents And Issues Associated With Sewage Sludge, Animal Manures, And Other Organic By-Products on June 4 - 6, 2001 in Cincinnati, Ohio. The purpose of the workshop was to review and discuss the effectiveness of by-products treatment and land application practices as they relate to the destruction, survival, and fate of emerging and re-emerging infectious disease agents given the available scientific information on the subject. The workshop reviews and discussions summarized the status of available data on the subtopics (bacteria, viruses, and parasites), identified gaps in information and provided suggestions to address the critically important gaps. The need for data inputs to address specific microbial risk assessment approaches being applied was acknowledged as an important, underlying commonality.

Considerable portions of the presentations and subsequent discussions were devoted to the state of the analytical methods and techniques used for sampling, detection and quantification, relative to the demonstration of disinfection by various treatment technologies. Supporting details about the microorganisms — including their fate and transport, appropriateness of indicator organisms, and comparative advantages and disadvantages of various treatment technologies used in the U.S. and Europe — are provided in the corresponding chapters that follow. Several presentations described how disinfection and environmental data are used to conduct microbial risk assessments, quantitatively, semi-quantitatively, or qualitatively, in either a static or dynamic context (i.e., accounting for host immune status and development of secondary infections). Finally, the critical types of data that are needed for future risk assessments were described.

Individuals with extensive technical expertise in a range of relevant supporting subtopics (bacteriology, parasitology, virology, public health, veterinary science, risk assessment, regulatory policy, environmental and wastewater engineering) participated in the review and discussion sessions. Workgroup sessions involving 12 to 15 participants each followed a series of oral reviews and perspectives. These work sessions focused initially on ranking the infectious disease agents of greatest prevalence and concern, then on the construction of a matrix of the strengths, weaknesses, and critical gaps in knowledge, detection, and treatment technologies. The matrices summarized key aspects of the issues for each agent. The broader scientific and technical communities, as well as decision-makers, can use this information to evaluate and prioritize needs and to focus future research and development efforts. Discussion sessions were used to exchange ideas on how to address information gaps and unresolved issues and to suggest areas for potential research, development, and collaboration.

Elements contained in this volume include: 1) The peer-refereed subtopic reviews that formed the basis for the oral presentations at the workshop; 2) The discussion sessions (including the conclusions and recommendations of the individual workgroups); and 3) An overall synthesis and interpretation of the proceedings. As part of the manuscript revision process, following the peer review that took place in 2003, authors were asked to update (as needed) their papers with more recent scientific findings, references and data citations. Contributions are presented as chapters with accompanying discussion sections as follows:

1. Highlights, Insights and Perspectives
2. Introduction, History, and Regulatory Perspectives
3. Bacterial Pathogens:
    - Sewage Sludge Workgroup Discussion
    - Animal Manures Workgroup Discussion

4. Viruses
    - Sewage Sludge Workgroup Discussion
    - Animal Manures Workgroup Discussion
5. Parasites
    - Sewage Sludge Workgroup Discussion
    - Animal Manures Workgroup Discussion

6. Microbial Risk Assessment

7. Microbial Risk Management

8. Appendices (List of Workshop Participants, Glossary)

Emerging/Re-Emerging Pathogens

The terms "emerging" and "re-emerging" were used for infectious disease agents that were one of the following: 1) New strains of microbes; 2) Strains that recently acquired virulence factors; 3) Microbes that have been around for many years but have recently acquired new significance based on various factors.

The emerging or re-emerging status of these agents is dynamic because what is "new" can change rapidly between the time when it is initially reported, identified, and subsequently studied; other "new" agents are continually emerging. Since a similar pathogen workshop in 1983, examples of emerging or re-emerging organisms of concern include: Bacteria: *E. coli* O157:H7 (identified early on but not prominent as a pathogen of concern until more recently), *Campylobacter* spp., *Listeria*, *monocytogenes*, and *Helicobacter*; Viruses: Hepatitis A, Rotavirus, and Norwalk agents; and Parasites: *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Microsporidia*, *Balantidium*, *Giardia*, and *Entamoeba*. At the workshop, participants examined the treatment processes and the accompanying environmental conditions to which the emerging pathogens are exposed, to discern their relevancy across pathogens and time. Based on available information and current research, participants concluded that there is no inherent difference in the temperature tolerances of emerging or re-emerging pathogens which would indicate that treatment technologies involving exposure time at high temperatures and/or drying conditions, such as composting, thermophilic aerobic digestion, anaerobic digestion, heat drying, and pasteurization — when properly conducted and operated — would have any less capacity to inactivate or destroy bacterial, viral, and parasitic (protozoan and helminthic) agents. Treating sludge via a process proven capable of removing enteric viruses and helminth ova to below the detection limit remains the best way to insure minimal risk to public health; all the other pathogens of concern are reduced to below their detection limit and thus to an exceedingly low, essentially imperceptible concentration.

Research Needs

In response to the challenge to clearly identify what needs to be known, is known, and is not known, the separate workgroups identified three major areas as needing attention: 1) Detection methods improvement; 2) Treatment efficacy (ensuring technologies are operated as intended); and 3) Site restriction and land application management controls. Research into each of these areas will provide essential inputs to any current or future risk assessments conducted, as well as to guide improvements in management practices for treated sewage sludge and manures.

An important overall research need is better documentation of the presence of pathogens and other organisms in raw sewage sludge and their fate through the various treatment regimes — including their survival in or on the soil or on crops after land application of the treated sewage sludge. To augment such presence and survival data and to provide context and perspective, field verification of the efficacy of Class A and Class B treatment processes (including data to directly relate process controls to initial and final pathogen and indicator densities) is needed. The following is a summary of research needs identified (presentation order does not signify priority):

- Detection methods for all groups of infectious agents (bacterial, viral, parasitic – protozoan and helminthic) need to be improved, updated, standardized, and validated for treated and untreated animal manure and sewage sludge.

  - Robust molecular, immunological, and immuno-magnetic separation and culture (IMSC) techniques for detection of pathogens at acceptably low limits and increasingly greater degrees of sensitivity need to be included.
  - Assays for detection and quantification are now available for some water-borne protozoan parasites: *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Microsporidia*, *Balantidium*, *Giardia*, and *Entamoeba*. These — along with methods/assays for fecal coliforms, *Salmonella* spp., enteric viruses, and helminth ova — require standardization and validation for manure and sewage sludge matrices.

- Evaluate occurrence, survival, fate and transport of protozoan and worm/nematode cysts and viruses (or appropriate surrogates) relative to different treatment and land application scenarios, using best available, standardized, analytical procedures.
- Evaluate the potential for use of one or more surrogates or models to determine the presence and survival of infectious agents before and after treatment and land application of treated sewage sludge and manure.
  - Characterize the relationship between the behavior of the infectious agents and the surrogates.
- Determine the prevalence, survival, and fate of antibiotic resistance determinants in bacteria (pathogens and non-pathogens) in treated sewage sludge and in raw and treated animal manures applied to land to document the degree to which the resistance determinants are being restrained via the treatments.
- Plan and coordinate future research to maximize generation of data suitable for use in microbial risk analyses and risk management decision-making.
- Determine the fate and transport of bioaerosols from land application and/or spray irrigation of treated wastewater sludge and raw and treated manure (liquid and solids).
- Determine and validate the adequacy of site restrictions and management practices for land application of Class B sewage sludge.
  - Evaluate the potential for fugitive dust and aerosolization of pathogens relative to worker and off-site exposure.
  - Determine how well riparian buffers prevent pathogen transfer/transport off farms and land application sites.
  - Determine adequacy of harvest and grazing restrictions developed for land application of treated sewage sludge relative to pathogen fate and transport, and the utility of this analysis to land application of animal manure.

Accessibility of Information

This document represents the tangible effort of the participants and organizers to make the information and discussions publicly available in order to increase transparency, dialogue, and understanding of the science behind the issues. Summary tables are designed to make information in this report accessible.

Subsequent workshops and presentations since the 2001 workshop have confirmed discussions and conclusions in these papers. In addition, work is underway within the U.S. Department of Agriculture and the U.S. Environmental Protection Agency on several of the research needs identified. These include analysis of bioaerosols from land application of treated sewage sludge, improved detection methods, evaluation of the effectiveness of riparian areas in filtering pathogens, and investigation of the survival/transport of pathogens at the watershed landscape scale.

It is evident from the papers presented and the workgroup discussions that more research has been conducted on infectious disease agents in treated sewage sludge than animal manure. With increased focus on regulating confined animal feeding operations and food safety, increased research is being devoted to pathogen control in herds and farms, especially with treatment of animal manures prior to land applica-

tion. Although not all pathogens potentially present in sewage sludge would be expected to occur in animal manure (and vice versa), contemporary methods of treatment, land application, and pathogen detection used for these residuals indicates that the same principles, concepts, strategies, and approaches would likely apply. This means that research advances in agriculture relative to pathogen control in food safety and water quality projects, and in the municipal and environmental sectors relative to water and air quality, will likely benefit these major societal concerns.

SECTION I

Synthesis, Overview and Introduction

Highlights, Insights, and Perspectives On Infectious Disease Agents in Sewage Sludge and Animal Manure in the U.S.

Why A Workshop On Emerging Infectious Disease Agents and Issues Associated with Animal Manures, Biosolids and Other Similar By-Products?

Highlights, Insights, and Perspectives on Infectious Disease Agents in Sewage Sludge and Animal Manure in the U.S.

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Introduction

The purpose of this chapter is two-fold: 1) Highlight the core principles and findings from the 2001 workshop so that all readers — scientists and non-scientists alike — can easily access the major outcomes; and 2) Provide a historical, policy and regulatory framework to shed light on, and better understand, the significance of the core principles and findings. Chapter 1 is divided into two parts. Part I focuses on the major findings and how they relate to questions that the public, regulatory, and scientific communities have posed. Information is presented in a question and answer format. Part II includes the underlying principles and practices involved in treating wastes potentially containing infectious disease organisms; historical perspectives of how past practices and concerns influenced the establishment and implementation of current regulations and practices regarding infectious organisms; and finally, the critical role of process performance evaluation and oversight in an arena where public health is involved.

This chapter also incorporates some research findings and studies, as well as policy, regulatory, public health and public perception issues, which have emerged since the workshop was held in 2001. The opportunity to analyze and assess both the workshop outcomes and more current developments has resulted in a comprehensive assessment of the state of the knowledge of infectious disease agents in sewage sludge and manure relative to effectiveness of disinfection treatment practices.

Editor's Note: The authors of this chapter have chosen to use the term "treated sewage sludge" vs. biosolids, as the US EPA has not officially adopted the term "biosolids" in the Part 503 rule to refer to sewage sludge that has gone through a pathogen and vector attraction reduction treatment process. For the industry definition of the word biosolids, see http://www.engr.psu.edu/ce/enve/publications/biosolids.pdf

Historically, significantly more attention from a regulatory perspective has been paid to sewage sludge versus animal manures. As a result, there are comprehensive federal and state regulations for sewage sludge that include treatment of pathogens and vectors. No similar set of rules exists for manure. At the same time, a significant number of documented — i.e., symptoms and/or illnesses confirmed in a clinical setting — food and water-borne illness outbreaks have been traced back to contamination by animal manure (e.g., Beuchat 1996; Cassman 1996; Smith et al. 2004; Tauxe 2002) versus the absence of similarly documented outbreaks related to treated sewage sludge in the U.S. The lack of documented outbreaks cannot be taken to mean that absolutely no infectious diseases have ever occurred in association with land use of treated sewage sludge. However, the fact that so many manure-associated outbreaks have been documented may reflect the fact that most land-applied animal manure is minimally or not treated, and therefore still a concentrated source of many types of fecal pathogens.

This situation makes the knowledge gained from regulating the treatment of infectious disease agents in sewage sludge increasingly relevant to future regulation of infectious disease agents in manures. The commonalities between sewage sludge and animal manure — in terms of microbes, and technological ap-

proaches to treatment — provide a rationale to leverage funding and multidisciplinary expertise to jointly collaborate on complex studies that will fulfill needs in both sectors.

Part I. Major Findings, Critical Issues

Infectious Organism Considerations

Discussion sessions at the workshop centered on three inherently interdependent, yet discernible, aspects of infectious organism considerations:

- Pathogens: Identification, detection, quantification, tolerance to destructive factors, and indicator organisms;
- Treatment technologies as they are currently configured and managed; and
- Risk analysis for comparison of various treatment and management options.

Infectious organisms fall into three general categories — "traditional" (known), emerging (unknown or of recent significance) and re-emerging (known but with new significance). Much attention these days in the scientific literature and general media focuses on the category of microbes and pathogens known as "emerging." Microorganisms referred to as "emerging pathogens" include:

1. Bacteria: Enterohemorrhagic *E. coli,* such as serotype O157:H7, *Campylobacter* spp., *Helicobacter pylori,* and *Listeria monocytogene;* and antibiotic-resistant strains such as *Salmonella enterica* pathovar Typhimurium, especially DT104, and other antibiotic-resistance-bearing bacteria such as *Enterococcus faecium*.
2. Viruses: Coxsackievirus, Echovirus, Hepatitis A, Rotavirus, and Norwalk-like agents.
3. Parasites: *Balantidium, Cryptosporidium parvum, Cyclospora catayensis, Giardia lamblia, Microsporidium* spp., and *Toxoplasma gondii*.

Ranking The Organisms

At the June 2001 workshop, following the formal presentations on bacteria, viruses and parasites in sewage sludge and animal wastes, workshop participants were divided into six discussion groups. (Edited transcripts of these discussions accompany the chapters that follow — see Table of Contents.) Workgroups considered all categories of infectious disease organisms, analyzing issues that could pose threats to public health. They also analyzed organisms with regard to their susceptibility to stressors (i.e., natural die-off and disinfection treatment). Based on these discussions, each workgroup consolidated their information into a matrix that considered the availability and quality of existing data for each organism, response to treatment technologies and lethal stressors, vector issues, odors, endotoxin, and worker exposure (e.g., Chapter 5, Table 1; Chapter 7, Table 3). Similar tables (not all equally extensive, mostly because no data exist) appear in each workgroup chapter.

Evaluating existing and emerging infectious organisms helps to rate their public health significance based on factors such as infectious dose, hardiness, susceptibility to treatment, etc. The result is a grouping from highest to lowest significance, a valuable tool for regulators, the public, researchers and others to come to some understanding about which pathogens carry the most weight (e.g., ones that are the hardiest and most resistant to lethal agents, and where the infections could pose the most significant threat). For example, an endospore former like *Clostridium perfringens* is very hardy when in its spore stage, but of relatively low public health significance in terms of its infectiousness and overall negative sequelae, i.e., lack of long term residual effects on individuals who recover from infection.

Indicator and Pathogenic Microorganism Analytical Needs

The workgroups all agreed that it is impractical to test manure and sewage sludge for all possible pathogens. No single test is available, nor is one likely to be in the foreseeable future, that could address the presence, absence, viability, and infectiousness of all possible pathogenic organisms. There was also a general consensus that an all encompassing test is unnecessary. Rather, there is a need to focus on key microbes and detection methods for them. The microbiological methods for detection and enumeration of fecal coliforms, enterohemorrhagic *E. coli, Salmonella* spp., enteric viruses, and helminth ova in sewage sludge and manure require improvement in some instances, especially with regard to standardization (manure) and validation (manure and sewage sludge).

When evaluating treatment process effectiveness, the workgroups concluded that better documentation is needed of the presence of pathogens and other microorganisms in raw sewage sludge and manure — as well as their fate throughout the various treatment regimes, including their survival in or on the soil and/or crops after application. Field verification of the efficacy of Class A and Class B sewage sludge treatment processes (including data to directly relate process controls to initial and final pathogen and indicator densities) also will be useful in documenting treatments. Tests also should include the presence, movement, and content of any bioaerosols.

The Core Question

The main question addressed at the workshop was:

Are the existing treatment technologies adequate to disinfect sewage sludge and/or animal manure from all known pathogens, including emerging and re-emerging pathogens?

For infectious microorganisms (bacteria, parasites, viruses) found in sewage sludge and manure, the answer is YES! Workshop participants agreed that the existing treatment technologies adequately disinfect sewage sludge to the extent that they are operated as designed and in accord with state and federal requirements or guidelines. Fundamentally, the principles upon which existing treatment technologies operate are the same for emerging and re-emerging pathogens as they are for traditional pathogens. None of the emerging or re-emerging pathogens (bacterial, viral, parasitic) exhibit biological or survival properties uniquely different from those already known for infectious disease microorganisms. Furthermore, some of the same types of treatment technologies (lagoons, anaerobic digestion, and composting) also have been reported to successfully achieve comparable amounts of disinfection with animal manure. In reality, though, there is less experience with disinfection technologies and accompanying development and validation of pathogen detection methods for animal manure than for sewage sludge.

> Emerging bacterial, viral, and parasitic pathogens exhibit biological and survival properties and characteristics equivalent to those described for many other bacteria, viruses, and parasites for which disinfection technologies are effective.

The effectiveness of the disinfection technologies used to treat sewage sludge and manure is evaluated by the presence/absence of the most treatment-resistant organisms (presuming they are present in the raw, untreated material). Enteric viruses and helminth ova were selected as indicators of treatment effectiveness because they are regarded, by microbiologists working with sewage sludges, as the most treatment-resistant organisms, and they can be quantified. By destroying the most resistant microbial forms, it follows that all other less resistant pathogenic microbes will be eliminated by that same treatment process. No treatment process is designed to produce a sterile product because the complete absence of microbes in a nutrient-rich material invites a population explosion of the first microbes to recolonize, which might be the pathogenic species. The presence of competing nonpathogenic microbes provides a biological buffer that prevents an overwhelming restructuring of the microbe abundances (or populations) present in the material. Participants emphasized this point during the workshop.

The disinfection strategies used for sewage sludge incorporate a multiple barrier approach, which involves a disinfection process in conjunction with a vector attraction control process. The federal regulation for sewage sludge management (40CFR503) created two categories of disinfection based on the multiple barrier approach. Class A processes are designed to reduce pathogens and vector attraction to a nondectable level that is considered a minimal risk to public health. Class B processes must incorporate time for natural attenuation to occur (see bullet below) and are intended to result in the same nondetectable level offered by Class A treatment processes.

All approved Class A and Class B treatment technologies (see Part II, Historical Perspectives, for an explanation of "approved") rely on one or more of the physical, chemical, and/or biological vulnerabilities of the target pathogens. The principles underlying these strategies center on the following factors (alone or in combination):

- Drying in the ambient air or with supplemental heat, e.g., heat drying/pelletizing, or air drying
- Heating to a high temperature for specific periods of time, e.g., composting, thermophilic digestion, heat conditioning of sludge for improved dewatering

- Raising the pH for specific periods of time, e.g., lime or alkaline stabilization
- Starving for a period of time, e.g., storage, digestion
- Exposing to toxic material, e.g., ammonia
- Irradiating with beta rays or gamma rays
- Natural attenuating in the ambient environment (usually over an extended time period, during which exposure to extremes of temperature, competition/interactions with soil organisms including predators, UV irradiation, desiccation, toxicity, and starvation ensue).

With the last element, natural attenuation, negative biological factors directly impact survival of the target pathogens. This additional biological activity includes effects from one or more of the following factors:

- Competition: Struggle among microorganisms for the limited resources (nutrients, water, oxygen, space, etc.) available in their microenvironment.
- Predation: Consumption and digestion of microbes by other microbes that occupy a higher level in the food web, i.e., big bugs eat the little bugs.
- Hyperparasitism: Growth of a secondary microbe in or on the primary parasite or pathogen (e.g., a virus (bacteriophage) attacking a bacterium like *E. coli).*
- Antibiosis: Inhibition of bacterial growth resulting from the natural presence of antibiotics produced by other microorganisms in the soil, manure, or sludge.

Historically, the main treatment technologies used for manure rely on desiccation and natural attenuation. In many cases, this treatment happens "unintentionally," i.e., due to typical farm management practices for manure that created conditions for desiccation and natural attenuation and results in 10- to 100-fold reductions in certain pathogens. In the past few years, recently developed technologies for on-farm use have incorporated some of the other above-mentioned factors used successfully to disinfect municipal sewage sludge.

Key Questions And Answers

The overall positive response regarding ability of existing treatment technologies to disinfect these two major types of residuals raises a series of related questions. The answers, for the most part, apply to sewage sludge treatment, simply because manure does not fall under a similar set of regulations with regard to pathogen disinfection despite the fact that pathogenic bacteria, parasites, and viruses can be present in manure. Key related questions and answers follow:

How do we know treatment technologies are being operated as intended?

Sewage sludge management practices employed by a municipal wastewater treatment plant must be reviewed, approved, and/or permitted by the relevant state and/or federal authority. Part of this review process involves an examination of the proposed monitoring protocols to insure that 40CFR503, or if stricter, state regulations are followed. For example, a facility permitted for an anaerobically digested Class B product is required to monitor detention time and temperature during digestion and the reduction of volatile solids. In addition, facilities must, under penalty of law, certify that they are following the protocols required to meet the regulations for each of the facilities' required monitoring periods (varies from one to 12 times per year depending on size of facility). This certification and any monitoring data are submitted to the appropriate regulatory authority annually. Some federal and state authorities conduct periodic and unannounced inspections to determine compliance. The reduction of pathogens and/or indicator organisms is usually not monitored for a Class B-PSRP product, since process controls have been demonstrated to be a reliable way of insuring pathogen reduction. Some states require that additional information be collected and reported. Process monitoring is also a key function of the Environmental Management System (EMS) program for biosolids, recently created by the National Biosolids Partnership (www.biosolids.org). An EMS establishes critical control points throughout the biosolids "value chain" (i.e., from point of entry into the wastewater treatment system through ultimate disposition of the biosolids product) to help ensure that the system is operating as designed and intended. Operation of the disinfection technology at the treatment plant, as well as management practices at the land application site (including control of site access to enable natural attenuation of Class B biosolids to occur) are critical control/monitoring points in the value chain.

Assuming treatment technologies are being operated as intended (i.e. according to Part 503 regulations), then what are the differences in potential for exposure to pathogens from the various treatment alternatives — particularly as they relate to implementation of the management practices that accompany them?

Class A processes are designed to reduce pathogenic organisms below the detection limit, and as such they provide maximum protection against exposure of the public to potential pathogens. Class B treatment processes, on the other hand, are not intended to reduce pathogens below the detection limit. They require a subsequent natural attenuation step to further reduce pathogens. Consequently, Class B treatment technologies are also dependent on additional restrictions on public access, grazing, and harvesting to protect the public from exposure to potential pathogens. Local climate, method used to restrict access, whether cake versus liquid is applied, method of land application (surface or incorporation), etc. all must be factored into setting those restrictions such that natural attenuation can occur as intended under the Part 503 rule. An EPA document, "Control of Pathogens and Vectors in Sewage Sludge," provides a thorough review of 40CFRPart 503 Class A and Class B sewage sludge treatment processes (USEPA 2003).

The multiple barrier approach (with pathogen and vector attraction reduction) applies to both Class A and Class B materials. The goal is to keep the host and the infectious organisms apart. Thus in Class B treatment, for example, a process is expected to reduce pathogens by a factor of ten or more; and natural attenuation is intended to further reduce them. Incorporating or injecting Class B sewage sludge into soil provides a soil barrier between the host and the organism. Additional considerations regarding specific treatment alternatives are discussed in Part II of this chapter in sections on Class A and Class B.

What is the level of confidence (based on data points, etc.) that the properties of known pathogens and their response to treatment technologies can be extended to emerging pathogens and the destruction of new variants?

The destruction strategies involving, for example, time, temperature and pH control, are expected — based on experience with recently emerged variants of pathogens — to be as effective for new variants of pathogenic bacteria, enteric viruses, helminths and protozoans, i.e., they will succumb to lethal stressors in accord with Class A and Class B treatments. The Part 503 regulation incorporated a large margin of safety to account for variations in the tolerance limits of different types of pathogenic bacteria, viruses, parasites and helminths to the different disinfection strategies. For example, the time and temperature relationships for Class A, Alternative 1 are partially based on the Food and Drug Administration's (FDA) research with food and can be expected to provide at least 5 $\log_{10}$ units of pathogen density reduction (Willis et al. 2004). Studies in the United Kingdom have also corroborated the effectiveness of digestion for removing *Cryptosporidium* and *Giardia* (UKWIR 2002).

Information for the newly emerged enterovirus responsible for the SARS outbreak (severe acute respiratory syndrome) indicates that while it perhaps is a little hardier than conventional enteric viruses, it is expected to follow the fate of other enteric viruses that are eliminated by Class A processing, Class B lime treatment, or are reduced one or more $\log_{10}$ units with standard Class B (non-thermophilic) digestion processes.

If increasingly sensitive analytical methods detect pathogens at concentrations that previously were nondetectable (e.g. Salmonella spp.), does that mean there is greater hazard or risk?

Different detection methods do not equate directly to greater or lesser hazard or risk than do previous methods. The sewage sludge or manure that is analyzed is still the same material, and the number of reported illnesses (if any) from infectious organisms is still the same. The workshop participants noted that both the helminth ova and enteric virus methods have been greatly improved in recent years by inclusion of a requirement for use of both negative and positive controls along with the test sample analyses — thereby defining the detection ability of the analytical laboratory in terms of recovery efficiency. These methods are the best currently available according to the microbial workgroup specialists at the workshop (and are in accord with 40CFR503). Although improvements can probably still be made (simply because no method is perfect), the workshop consensus was that further improvements will take time and additional financial support for research.

The ability to increase the sensitivity of detection also must be balanced by the relevance of the measurements to what is known about the infectious dose. For example, with *Salmonella* spp., <3 per 4 g vs.

10 per 4 g may be statistically different but that difference is not practical if the total infectious dose were 10,000 organisms, as has been suggested in the contamination case of sprout consumption (Gill et al., 2003). A person would need to ingest 500 to 1000 g of total solids of treated sewage sludge or manure to achieve this dose; such an amount likely would not go unnoticed. In effect, increasing detection sensitivity helps refine our understanding of pathogen die-off/survival rates.

Related Questions

What is the current scientific research regarding air as a pathway of exposure?

Recent research conducted by the University of Arizona (Brooks et al. 2004) involved collecting air samples from land application sites around the country while treated sewage sludge (in both liquid and cake form) was being applied. The reported findings suggest that pathogens are not present in air at infectious dose concentrations. EPA also is sponsoring some field research with regard to bioaerosols and land application of treated sewage sludge. Other published reports (Gattie et al. 2004) raise public health issues with regard to airborne pathogens that this additional research is expected to address.

An effort was made in the 1980s to do a quantitative microbial risk assessment of pathways of exposure with the application of treated sewage sludge to land. Such an approach was ultimately not used in establishing 40CFR503 because of insufficient data on the presence of microbes and associated dose-response information. A report on this effort (Kowal 1994) suggested that the most exposed individuals are the sewage sludge workers. It speculated about what organisms might move off site, and since parasites, relatively speaking, are big and bulky, they will not move into air, won't remain suspended in water, and will be captured in the soil or foliage. Bacteria also adhere to solids, such as the soil, and as such will not go far. Organisms that appeared to have the ability to move into groundwater and air are the viruses. However, viruses are very readily sorbed to soil (because they are highly charged) and, except in coarse textured soils, aren't likely to percolate downward. In general, because these organisms adhere to soil, the greatest risk of exposure is by direct ingestion.

NIOSH's (2002) guidance on worker safety relative to Class B sewage sludge focuses on three areas: 1) Basic hygiene recommendations for workers; 2) Provision of appropriate protective equipment, hygiene stations, and training; and 3) Good environmental practices to prevent and minimize occupational exposures.

Endotoxins are a microbial product (not an organism but parts of an organism) that when dried and pulverized to micron and submicron size particles may become airborne (i.e., as a bioaerosol) when material is agitated. Endotoxins are ubiquitous in the environment therefore any measurements related to land application of manure or treated sewage sludge need to take into account ambient levels. The size of the bioaerosol as it reaches susceptible receptors downwind of a land application site would be an important characteristic to measure. Particles too large to enter and deposit in the upper or lower airways would not be expected to elicit a respiratory response, as would be typical for respirable endotoxin or endotoxin-containing dust. Baker et al. (1986) report that endotoxin concentrations larger than 0.8 micrograms/m^3 can lead to decreased lung efficiency; 0.1 micrograms/m^3 can cause Organic Toxic Dust Syndrome.

Arid, windy areas that receive manure or treated sewage sludge should be studied to determine the concentration of airborne endotoxins (once ambient levels are determined) after applied material has air-dried and is subjected to wind gusts. The pulsed nature of wind gusts sufficiently energetic to dislodge persistently increased concentrations of endotoxin-containing particulates from manure or treated sewage sludge is expected to be very infrequent. More dispersion as gusts move downwind from the emission point would clearly reduce the airborne concentrations even further. Threshold amounts of endotoxin for even minimal response on a continual 8- and 10-hour (workshift) exposure are available from published reports (Castellan et al. 1984; Michel et al. 1997, Millner et al. 1994). These would provide a threshold response level against which to compare measured or predicted concentrations (from dispersion models) downwind of an application site under various atmospheric conditions. By including data obtained concurrently on emitted concentrations of volatile odorous compounds and particulates with microorganisms, it would be possible to know the simultaneous exposure to several types of airborne constituents that may potentially contribute to complaints by neighbors in the vicinity of manure or treated sewage sludge land application areas.

For bioaerosols to pose a public health risk, they must get into the air. Thus management practices — not the disinfection technology — are the critical variables to control from a public health perspective. In the case of land application practices, the "delivery mechanism" for putting the treated sewage sludge

or manure on the soil is a critical control point (i.e., using direct surface application or injection vs. an irrigation gun when public health impacts from bioaerosols are a consideration). Incorporating or injecting treated sewage sludge or manures into the ground almost totally eliminates the risk of bioaerosols being produced.

To what extent is antibiotic resistance a problem in farm animal manures vs. sewage sludge as has been found and reported in the U.S. and Europe?

Development of antibiotic resistance in pathogenic microorganisms is a very important issue among public health specialists as evidenced by recent efforts worldwide to curtail the overuse of antibiotics in clinical settings as well as in veterinary and food-animal rearing operations (WHO 2001). Considerable international debate still persists about the role of antibiotic use in agriculture, particularly for growth promotion in food animals, and the risks associated with development and amplification of antibiotic resistance (Turnidge 2004). Experts agree that ample data show a substantial development of resistance against many classes of antibiotics used in both food animal production and in treatment of human diseases, especially among *Campylobacter* spp. and *Salmonella* spp. and non-pathogenic *Escherichia coli* and *Enterococcus faecium*. However, disagreements about the rates of transmission of resistant bacterial strains, or resistant genes, to humans from food animals (directly or indirectly), and the extent of the harm, if any, are major areas of contention (Phillips et al. 2004; Turnidge 2004). The U.S. Food and Drug Administration (FDA) and the European Union (EU) have adopted totally different approaches to dealing with these disagreements. The FDA requires proof that a problem has actually emerged (such as it did in withdrawing approval of a fluoroquinolone from poultry (FDA-CVM 2000)), whereas the EU disallows usage of all feed-based antibiotics when the primary purpose for their use is "growth promotion" rather than infection control. (For example, use of non-growth promotion fluoroquinolones in food animals is allowed.)

In an effort to infuse the debate with more objectivity, Salisbury et al. (2002) and Turnidge (2004) have highlighted the need for a contemporary, transparent, risk assessment-cost benefit analysis of this complex issue. Salisbury et al. (2002) presented a convincing case for a tri-partite risk analysis approach that identified antibiotic use, environmental spread, and genetic transfer relative to animal and human health as major elements analogous to chemical, microbiological, and genetic risk assessments, respectively. Salisbury et al. (2002) also described how an Australian group of experts (JETACAR 1999) proposed to coordinate this tri-partite approach into a risk management program. Presently, no national or international agencies with responsibility for animal or public health have developed a comprehensive risk analysis or long-term management program for antibiotic resistance. In terms of land application of manures and treated sewage sludge in the U.S., to the extent that treatments to reduce the numbers of microbes have been successful, there will be a concurrent decrease in those parts of the microbial population that have acquired antibiotic resistance. As is well known, once the presence of the antibiotic diminishes, the pressure to maintain the resistance elements decreases and there is a natural attenuation in this trait in the wild, e.g., in the soil to which the animal manure or treated sewage sludge has been applied. Thus, while there is still no conclusive answer to this issue, evidence thus far available suggests that the soil environment is not very supportive of continued amplification of antibiotic resistance factors in pathogenic microorganisms.

Have there been any new research developments regarding exposure to BSE via sewage sludge?

Disinfection principles and concepts have been challenged with regard to prions, the infectious self-converting abnormally-configured proteins that cause TSE (transmissible spongiform encephalopathy), e.g. in the United Kingdom, B.S.E. (Bovine Spongiform Encephalopathy) or Mad Cow, in the western U.S., chronic wasting disease (or Scrapie in sheep). Information on prions is still being developed and evaluated including its fate in wastewater treatment and sewage sludge processing. The topic of BSE was discussed at the workshop. It was agreed that prions, which are not microorganisms *per se*, appear to resist complete degradation by most treatment technologies except pressurized alkalinolysis or incineration. The United Kingdom's primary approach to control BSE has been to manage what cows are fed (ruminant feed ban) and require that the parts of cattle and sheep most likely to carry BSE or scrapie agents (brain tissue, spinal cord) be removed from any slaughtered animal that will be consumed by humans or bovine stock. The exposure to humans via the consumption of vegetables grown on soil receiving sewage sludge in the UK was calculated to produce an annual risk of nvCJD (the human form of mad cow disease) of 1.0×10^{-7} cases per person (Gale and Stanfield 2001; Gale 2002).

To what extent are animal viruses a concern to humans?

The issue with SARS (severe acute respiratory syndrome) raises a more general question about whether animal viruses are a concern to humans. As more is learned about SARS, there is some indication that

the virus may have jumped from animals to humans (Bush 2004; Osterhaus et al. 2004). Such crossing of the species barrier, especially by viruses and protozoans, is one of the important projections that has been made about future prospects of emerging diseases (Kay and Pringle 2004; Taylor et al. 2001; Woolhouse 2002). Overall, as the Sobsey chapter in this publication indicates, there are rare examples of animal viruses that are infectious in humans. A notable example is type A strains of avian influenza, which cause an infectious disease in birds. Although this disease has a global distribution and has been known for more than 100 years, it can be regarded as emerging because of its very high rate of genetic mutation, which fosters its ever increasing virulence (Li et al. 2004). In 1997, Avian Flu Virus subtype H5N1 passed from birds to humans, resulting in the death of six of the eighteen people who were hospitalized with severe respiratory disease. Subsequently, between 2001 and 2004, as a consequence of genetic rearrangements in the virus, additional outbreaks occurred. In 2004, the first case of human-to-human infection was confirmed (Li et al. 2004). Pigs (Webby et al. 2004) and cats (Kuiken et al. 2004; Thornley 2004) are now known to be susceptible to some strains of human as well as avian flu viruses. Thus, there is concern that these animals and possibly others eventually may become sources of emerging strains (Karasin et al. 2004).

In addition to spreading in water droplets, the avian flu virus is known to be shed in feces. Thus, bird droppings — or water contaminated by wild bird droppings — bird feet and feathers, and infected or contaminated animals or water could contribute to dissemination of the virus. The presence and susceptibility of the disease in domestic and wild fowl (Campitelli et al 2004), the concentration of poultry production and live animal markets in the Asian region (Webster 2004), and free-ranging production practices that allow wild and domesticated birds to intermingle, make control of this infectious agent a world health concern (Ferguson et al 2004). At present, because animal vaccination is a controversial issue for this virus, it is used only in some countries. No vaccine currently available can fully protect humans from the most virulent subtypes of the avian flu virus. However, existing treatment technologies for manure and sewage sludge — when operated as intended — will inactivate these special strains as their biochemistry makes them susceptible to the same lethal agents as other strains that succumb to waste treatment.

What research needs to be conducted regarding animal manure to allow for informed decision making regarding regulation development?

Research needs identified for animal manure include:

- Completing a manure-related infectious disease incidents database and including the several well-documented cases that resulted from contamination of water or foods with fecal microbes from farm animals/environments;
- Identifying applicable treatment technologies for reducing pathogens (and possibly vector attractiveness) from experiences with agricultural, industrial, and municipal residuals (for example, anaerobic digestion, aerobic digestion, lime treatment, composting);
- Developing information regarding the degree to which food- or water-borne illness pathogens can survive in manure-treated soils or migrate onto harvestable plant parts; and
- Preparing a guidance manual for meeting requirements of the confined animal feeding operation (CAFO) regulations.

Processes with the potential for reducing the pathogen content of animal wastes like anaerobic digestion, aerobic digestion, and storage are expected to accomplish a 1-2 $\log_{10}$ reduction in pathogenic microorganisms like *E. coli* O157:H7 and *Salmonella* spp. Treatments that involve thermophilic composting, thermophilic digestion, or alkaline pH can be expected to accomplish at least 3-4 $\log_{10}$s of reduction when conducted properly (Cassman, 1996).

Part II. Historical, Regulatory and Public Policy Framework

Historical Perspectives

Until the early 1970s, sewage sludge in the U.S. often was applied with minimal treatment and at very large loading rates to agricultural lands. Despite this mode of management, published reports of water- and food-borne illness outbreaks traced back to these practices in the U.S. are not readily found in the literature. Dilution of wastewater by direct discharge to rivers and streams was frequently viewed as the primary means of dealing with pollution. That practice continued until dense urban populations and large industrial discharges made it untenable. For centuries, raw sewage from European cities was land applied/used in agriculture.

During this same period, land application of animal manure was being done on small to modest size farms much as it always had been, with little or no prescriptive guidance or regulation. Frequently the farmer stored the manure in a pile or in a basin for a period of time, often the winter, before using it on the farm. This storage may have accomplished some disinfection, but the amount was mostly limited and highly variable within and between operations, and hence unreliable.

In the 1970s, several concerns surfaced with the practice of applying effluents and residuals to land: 1) Eutrophication in lakes, rivers and streams; 2) Contamination of groundwater; 3) Phytotoxicity; 4) Infectious disease issues, and 5) Heavy metal contamination of the soils. These concerns led to collaborative research being supported by federal agencies in the U.S. to address issues like agronomic application rates for nutrients, the cation exchange capacity of soils, and treatment to protect human health from any pathogens that might be present. The agencies included the Food and Drug Administration, the U.S. Department of Agriculture, the U.S. Army Corps of Engineers, and the U.S. Environmental Protection Agency. In 1979, for the first time, a federal regulation — 40CFR257, Criteria for the Use or Disposal of Solid Wastes including Sewage Sludge — addressed human health issues related to infectious diseases and the pathogens in sewage sludge. One intent of this rule was that a working relationship be established between the landowner and sewage sludge generator, recognizing that the landowner needs a certain quality product (e.g., plant nutrients and micronutrients but not infectious levels of pathogens or heavy metals) from the generator. Open and frequent communication between these two parties was an important ingredient for a successful outcome.

During this period of study and regulatory inception for sewage sludge, animal manure application on land remained virtually unregulated.

Part 257, Part 503 and Pathogen Reduction Requirements

It took time after adoption of 40CFR257 for states to create the infrastructure for implementing regulatory and oversight programs as outlined, including permitting of sewage sludge management programs. During that time, however, many states took advantage of EPA construction grant funding to help with installation of sewage sludge management systems, including anaerobic digesters and composting operations.

Infectious disease control processes employed today — PSRP (Processes to Significantly Reduce Pathogens), PFRP (Processes to Further Reduce Pathogens) and VAR (Vector Attraction Reduction) — were largely created within the Part 257 rule. Both PSRP and PFRP required a VAR step. PSRP processes were required to stabilize sewage sludge to minimize attractiveness to vectors as well as reduce pathogens. PFRP processes were expected to reduce pathogens below the detection limit and reduce attractiveness to vectors. Approved treatment technologies in Part 257 included: anaerobic digestion, aerobic digestion, lime stabilization, pasteurization and composting.

As new treatment technologies for sewage sludge disinfection were developed, state regulators and U.S. EPA needed a way to affirm that these technologies were equivalent to those in Part 257. Thus in 1985, EPA established the Pathogen Equivalency Committee (PEC) as a "recommending body" to make determinations about the ability of new technologies to remove pathogens equivalent to Part 257 PSRP or PFRP technologies. The PEC uses a rigid evaluation process to determine removal of enteric viruses (3 logs) and helminth ova (2 logs) and also evaluates the qualifications of the laboratory and analysts to do the work. Wastewater treatment plants must demonstrate the new technology's equivalent ability to that of existing PSRP or PFRP technologies to remove pathogens. The permitting authority usually bases granting of equivalency on the recommendation of the PEC. Over the years as many as 20 disinfection technologies have been recommended for PFRP equivalency (national and site specific) via this approval process (EPA 2003). The number of new technologies in the pipeline has shown a rapid increase in the past couple years.

In 1993, EPA promulgated the 40CFR503 sewage sludge regulations, which replaced the Part 257 rule relative to minimum federal requirements on sewage sludge use and disposal requirements. Like Part 257, the Part 503 regulations contain the PSRP and PFRP disinfection processes. The public access and harvesting restrictions are still the same as under 40CFR257. Vector attraction reduction was always viewed as a necessity. The methodologies for achieving it (reducing volatile solids, reducing oxygen uptake, desiccation, and employing injection or incorporation to place a barrier between the treated material and people) were initially more or less included in the PSRP and PFRP process descriptions. However, the options available for VAR implementation were not clearly identified and spelled out in regulatory language until 40CFR503 was adopted.

The main differences are in Class A, Alternatives 1, 3, and 4 and Class B, Alternative 1. Class A, Alternative 1 is an expansion (to the form of equations, making allowances for the solids concentration) of what was known about the efficacy of the time-temperature relationships in 40CFR257 relative to pathogen destruction, e.g., by composting or pasteurization.

The responsibility for implementing 40CFR503 primarily rests with the USEPA; states can seek authorization from EPA to implement the rules within their jurisdiction. To date six states — Arizona, Oklahoma, South Dakota, Texas, Utah and Wisconsin — have been authorized to administer the program. EPA would like to authorize the remaining states and territories to implement the rules, but many of them have encountered legislative and resource hurdles that impede authorization. Essentially all of the states have adopted requirements as or more restrictive than the Part 503 requirements.

Changes in Sewage Sludge and Its Management Over 30 Years

Anecdotal evidence has suggested that sewage sludge composition in terms of quantities and types of microorganisms has significantly changed over the years. For example, the numbers of *Salmonella sp.*, enteric viruses, and helminth ova usually found in sewage sludges in the year 2004 are much lower than they were 30 years ago. Between 1940 and 1980, microbial loads in primary sludge averaged 2.0×10^7 fecal coliforms per g dry weight; 4.1×10^2 *Salmonella* per g dry weight; enteric viruses averaged 400 plaque-forming units (pfu) per g dry weight; ova of Ascaris ranged from <1 to 50 ova per g dry weight (USEPA 1981).

By 1994, untreated sludge from a large city in the central region of the U.S. showed similar levels to those mentioned above for fecal coliforms, but reported 75 percent less for *Salmonella* (23 per 4 g); 100-fold less for enteric viruses (17 pfu / 4 g); and >100 percent less for helminth ova (4.2 per 4 g). In 2003, another large U.S. city reported that helminth ova were 1 or less per 4 g dry solids and enteric viruses were 2 to 20 pfu per 4 g dry *untreated* sludge solids. Given these indications that untreated sewage sludges have significantly changed in pathogenic characteristics, an up-to-date survey of untreated and treated sewage sludges is necessary if any changes to current pathogen regulations are to be made in the future.

> Microbiological quality of untreated sewage sludge has significantly changed for the better in many municipalities over the last 30 years; the numbers of enteric viruses, helminth ova and salmonella have decreased significantly.

Over the past 30 years, there also have been changes in the way sewage sludge is physically handled at some facilities; shifts from a liquid to a dewatered solid or cake product has occurred in some situations. This shift, in turn, has led to differences in how the sewage sludge is treated. For example, lime stabilization is now accomplished by either adding lime to liquid sludge or to sludge cake.

The use of lime, at least in the U.S., originated with the addition of $Ca(OH)_2$ or hydrated lime in a slurry to a liquid sewage sludge; the process was known as lime stabilization, currently a Class B, PSRP process. Similarly, all of the original work with pasteurization, largely in Europe, was done with heating of a liquid sludge either with heat exchangers or by steam injection. It was not until several years after the promulgation of 40CFR257, and following research in Europe and North America, that the idea of adding CaO, quick lime to dewatered sewage sludge to both evaporate water and raise the sludge's temperature to the pasteurization level and beyond was introduced. Almost immediately, the treatment industry saw what appeared to be a simpler and more economical approach to both achieving pasteurization and gaining the VAR benefits of lime stabilization. They also reasoned that if quick lime (CaO) could be mixed in large quantities with sludge cake, they also could mix hydrated lime ($Ca(OH)_2$) in much smaller quantities with sludge cake and achieve lime stabilization. Both lime treatment approaches with cake, i.e., stabilization and pasteurization, meant handling much less water.

Transition to More Distant Management

The approach taken today with application of treated sewage sludge to land differs significantly from the earlier days. In the 1970s, most sewage sludge — untreated or minimally treated — was applied as a liquid slurry to fields located near the POTW or dewatered via sand drying beds and given away to local landowners. Today a large amount of treated sewage sludge is mechanically dewatered to reduce transportation and/or storage costs and land applied to sites located a considerable distance away from the POTW. Furthermore, since the 1970s, many utilities have contracted out transport and application operations. While these steps may have simplified the tasks for the utility in some respects, they also have distanced the generator from the landowner who needs to specify the kind of product needed (e.g., nutrient content,

physical properties) and the application method preferred (e.g., surface applied, incorporated, or injected). As the direct connection between the landowner and the generator evolved into one that included an intermediary — the land application service company — the on-farm oversight of management practices became less influenced by the generator and more subject to their contractors' adaptations. Increasingly, many wastewater treatment plants rely on the intermediary land application service contractor for multiple, associated operations, e.g., storage at the treatment plant, dewatering, transport, storage in the field, deciding where and when to spread, field testing to determine application rate, permitting specific fields, and public information and community relations.

Despite this trend toward contracting out/privatizing multiple services related to treated sewage sludge management, the Part 503 rule states that the preparer of the sewage sludge (typically the treatment plant) is legally responsible for its ultimate disposition. This legal responsibility — especially in light of more distant land application sites and processing facilities and the contracting out of management services — drives home the need for wastewater treatment plants to establish oversight and monitoring for its treated sewage sludge recycling programs. Some states have adopted their own oversight requirements that more directly keep the treatment plant in the loop. For example, the New Jersey Department of Environmental Protection has taken a "cradle to grave" regulatory approach in some instances, specifying everything from how sewage sludge is managed (including odors) at the treatment plant, to how it is transported, how it is stored in the field, how it is applied, etc. The National Biosolids Partnership's EMS program also lends itself to more oversight and monitoring, as do some local governments' ordinance overseeing land application of treated sewage sludge.

Current Regulatory Structure and Concerns

The 40CFR503 Class A pathogen disinfection requirements are designed to reduce the level of pathogenic organisms in the treated sewage sludge to below the detection limits of the analytical methods specified and thus be maximally protective of public health. Thus, the possibility of pathogen concentrations being present in amounts typically needed to provide an infectious dosage is highly unlikely. As noted earlier, for Class A, the time and temperature treatments are very conservative, allowing for more than a five-log reduction of pathogens. For example, with anaerobic digestion as a Class A process, the design engineer following Class A, Alternative 1 needs to plan on holding sludge in a batch condition for 24 hours at 55°C to meet that conservative limit. As noted in Part I, workshop participants stressed the importance of recognizing that disinfection is not equivalent to sterilization; nonpathogenic microorganisms are expected to be present in the final product to contribute to microbial buffering, competition, and decomposition.

When the Class B requirements are met, workshop participants noted that the level of pathogenic organisms has only been significantly reduced. Thus, the product needs to be managed in a way that limits public and domestic animal contact with it, until further decline in pathogen populations can occur. These precautionary measures involve restricting the public's access to the land application site, controlling animal grazing, and prevention of crop harvesting for specified periods of time after application. With field applied sewage sludge, this further decline occurs with time as a consequence of natural attenuation. During the attenuation period, proper management and handling are necessary; this is what the field management practices and access restrictions are intended to provide.

In contrast to the regulations for the land application of sewage sludge, the workshop participants noted that currently there are no federal regulations requiring treatment of animal wastes or manure to reduce pathogens before application to land. Yet, the total annual production of manure from animal feeding operations is estimated at about 500 million tons ('as excreted'), 300 million of which come from confined animal feeding operations or CAFOs (Federal Register Notice, 2003). (In comparison, less than 10 million dry metric tons/year of sewage sludge are generated.) Indirectly, animal manure applications to land are controlled by the CAFO and Total Maximum Daily Load (TMDL) rules. The CAFO rule ((68FR7176), issued by EPA in December 2002, forbids discharge of animal wastes from large CAFOs (i.e., those with more than 1,000 animal units) to surface waters and requires a nutrient management plan to be in place when manure is spread on land owned by the CAFO. Unfortunately the rule does not address manure that is exported from the site, nor does it directly address the issue of pathogens. The only way that pathogens might be addressed is, for example, if the water quality standards for fecal coliforms in a stream near a CAFO were exceeded and the situation clearly was attributable to manure from the CAFO. The TMDL rule, which is concerned with water quality standards, would then require changes in how animal waste is managed.

Indicators As Measurement Tools For Pathogens

With the existing treatment methods, workshop participants agreed that all sewage sludge should be treated by a proven process even if its initial indicator/pathogen load is determined to be below the treatment standard limits (e.g.

Class B Alternative 1; see discussion below on Class B disinfection,). Examples of Class A PFRP processes include heat drying, pasteurization, and composting, which have been demonstrated to reduce pathogens to below the analytical detection limit. For Class B, PSRP processes like anaerobic digestion, aerobic digestion, and lime stabilization have been demonstrated to reduce fecal coliforms by ≥ two $\log_{10}$ units and pathogens like *Salmonella* spp. by ≥ 1 $\log_{10}$ unit, thus the stipulation for use of field management practices and access restrictions where further pathogen attenuation occurs. It is the latter stipulation that highlights a fundamental concern that the public expresses about land application of treated sewage sludge: the need for some assurance that specified regulations for treatment, vector attraction reduction, and access restrictions are being implemented consistently.

Recognition of this public concern led to a discussion at the workshop about the impact and significance of using indicators as a measurement tool in pathogen testing. It was noted that the term "indicators" as used in association with treated sewage sludge and animal manure is different from the meaning when applied to drinking and recreational waters. Indicators serve several purposes with sewage sludge and manure management, as outlined below:

- They can be an indication of contamination by animals (including humans), of which some may be ill.
- They can be used as an indication of treatment effectiveness.
- In the case of Class A biosolids, *Salmonella* and/or fecal coliforms can be used to show that treatment has occurred and the product is not contaminated.
- Fecal coliforms, especially ***E. coli***, primarily serve the purpose of:
  - Indicating human fecal or animal waste contamination
  - Showing that the material was or was not effectively treated and/or improperly handled after treatment, thus allowing product contamination to occur, as with Class A, and the 'regrowth' issue (see Yanko chapter for details).
- Enteric viruses and helminth ova are used (primarily by the PEC) to determine the effectiveness of treatment processes. Since they often are not present in substantial numbers in U.S. sewage sludges, they must be added/seeded to the sludge being treated when a process is being evaluated.

Insights Into Class A Disinfection For Sewage Sludge

Many facilities approach a process for reducing pathogens in sewage sludge below the detection limit (e.g., 40CFR503, Class A, Alternative 1, time and temperature) without realizing it was derived from experience with fluids. So, for safeguarding public health, it is important to demonstrate that all sludge particles have been subjected to a proven treatment process. It is relatively easy to mix a fluid material, like a liquid sludge, but more difficult to mix a sludge cake. Similarly, it is easier to pasteurize milk than to pasteurize the milk after it has been mixed with polymers, stabilizers, and thickeners to form a cake batter. The central issue is ensuring that all (even very small) particles are exposed to the stressor(s) (e.g., temperature, pH, heat, ammonia, etc.) for the same total amount of time required to meet a threshold criterion. With a fluid or liquid, the freedom of movement of even the small particles throughout the treatment enclosure affords them the chance to reach the exposure criterion threshold — even in an environment in which temperature gradients, for example, might exist. Operationally, increased mixing and retention can be used to ensure adequate exposure. On the other hand, with a very viscous paste the opportunity for very small particles to move into a treatment temperature threshold zone is limited until major amounts of energy are expended in mixing or ensuring that heat transfer to all zones is adequate.

Class A, Alternative 1 relates the necessary temperature to be achieved to the time it must be held given a specified solids concentration to insure adequate disinfection. When a facility submits a plan to the permitting authority, the monitoring plan must show how the operator will insure that all parts of the sludge meet the appropriate time and temperature requirements before discharge from the disinfection process. In general, this alternative is limited to batch or plug flow reactors and is not available to continuous flow reactors because of possible short-circuiting (i.e., bypassing full treatment time-temperature).

Class A, Alternatives 3 and 4 require the utility to determine the quantities of enteric viruses and helminth ova in the sewage sludge before and/or after some form of disinfection processing to show that they

are present in quantities below the detection limit after treatment. These alternatives are mostly used for poorly defined processes and have several limitations and/or concerns. The difficulty with Alternatives 3 and 4 is that enteric viruses and helminth ova are misused as indicators of the presence or absence of all pathogenic organisms. Enteric viruses and helminth ova are *solely meant* to be indicators of treatment efficacy, not indicators of overall microbiological quality of the product. Results are subject to misinterpretation because it is presumed that the treatment facility is handling sewage sludge with substantial numbers of enteric viruses and helminth ova and thus reductions could be traced through the treatment process. If none are detected in the untreated sewage sludge, then Alternatives 3 and 4 are meaningless options and the treatment plant should select a different treatment method. The preferred approach is to demonstrate that each treatment technology, when operated within certain and specific limit criteria, is adequate to disinfect the sewage sludge based on the most resilient pathogens, i.e., enteric viruses and helminth ova (as specified in the "White House" document — available at www.epa.gov/ttbnrmrl/ and scroll to Septage/ Biosolids/Sludge).

> Enteric viruses and helminth ova are very disinfection-resistant pathogens, and therefore are used as the primary biological indicators of process and treatment efficacy when a disinfection treatment process is initially undergoing evaluation by the US EPA – Pathogen Equivalency Committee. They are not indicators of overall microbiological quality of the product.

The three processes most frequently used for meeting Class A, Alternative 5 - PFRP criteria are composting, pasteurization, and heat drying. With composting it is critical that all parts of the sewage sludge pass through a zone where they can be held for no less that 3 days at 55ºC and then be removed without contamination. Pasteurization refers to holding the temperature of a fluid at 70ºC or above for at least 30 minutes. (This is a good example of treatment quality as specified by the Part 503 rule.) Achieving the equivalent results when mixing a powder, such as quicklime, with a semisolid material, such as sewage sludge, is not easy by any means. The mixing has to be thorough enough to insure that all parts of the sewage sludge come into contact with the alkaline material *and* that the temperature gets elevated to 70ºC or above *and* is held there for at least 30 minutes. A minimum amount of water needs to be present and available for reaction with the calcium oxide. These systems must be carefully designed, tested, and monitored (for dosage, mixing, moisture level, pH, odors) to insure that they are operating properly.

In evaluating the equivalency of new processes to Class A, Alternative 5, the PEC recommends that there be at least 100 helminth ova and 1,000 plaque-forming units (pfu) of enteric viruses per 4 grams of sewage sludge entering the treatment process. This facilitates a determination of whether or not the process is capable of achieving ≥3 $\log_{10}$ units of reduction in enteric viruses and ≥2 $\log_{10}$ units of reduction in helminth ova. Enteric viruses and helminth ova are regarded as the most treatment-resistant organisms, thus, when conditions of a treatment process result in their destruction, it has been shown that the other less resistant pathogenic agents will be eliminated as well.

Insights Into Class B Disinfection For Sewage Sludge

Requirements under 40CFR503 provide two alternatives for sewage sludge to be categorized as Class B with respect to pathogens. As noted several times in this chapter, when treated sewage sludge is land applied these alternatives have to be combined with access restrictions to allow for natural attenuation to occur. Only after natural attenuation can the material be considered a minimal risk to the public. Alternative 1 tests for the number of fecal coliforms present; Alternative 2 uses a PSRP process like anaerobic digestion, aerobic digestion, lime stabilization, etc. Alternative 1 is satisfied by periodically monitoring the processed sewage sludge for fecal coliform density. If the geometric mean of at least seven samples of treated sewage sludge is a MPN or cfu of ≤2,000,000 fecal coliform per gram of total solids (TS), the sewage sludge meets Class B disinfection requirements. The major difficulty with this criterion, however, is that many raw sewage sludges do not have more than 2,000,000 MPN fecal coliforms per gram of TS prior to treatment. Thus, the presence/absence of fecal coliforms neither provides adequate information about how the sludge was processed nor about the presence of pathogens. Therefore, participants in the Bacteria-Sludge/Biosolids workgroup (see Chapter 4) found this criterion to be of no value, noting that "many sewage sludges can be classified as Class B on the basis of their fecal coliform concentration without having undergone treatment."

For PSRP process testing, EPA continues to expect a 2 $\log_{10}$ reduction of fecal coliforms and ≥ 1 $\log_{10}$ for pathogens like *Salmonella* spp., viruses, and parasites. This also is expected for those microbes distin-

guished as emerging pathogens. As noted above, what happens in the field to the sewage sludge following land application is critical, since natural attenuation is considered part of the overall disinfection process with Class B material and it is intended to reduce any remaining pathogens to a level below the detection limit. The shortage of follow-up data after land application made it difficult for the workgroups to discern to what extent the field management requirement(s) were met or exceeded.

Insights Into Vector Attraction Reduction

One of the options for meeting the vector attraction requirements with a biological digestion process is to show that volatile solids were reduced by 38 percent. Regrettably, it is difficult to use a single number for all raw sludges undergoing digestion in the U.S. Wastewater differs throughout the country, depending to some extent on whether the municipality has a separate or combined sewer system for its municipal sewage and storm water. As such, the organic content of the sewage sludge and the volatile solid reductions that can actually be achieved will vary from under 30 percent to over 60 percent. This variability points to the need for the required volatile solids reduction to be tied to the characteristics of the sewage sludge being digested, i.e., recognizing that a set number like 38 percent is not universally applicable. As a general practice, to limit dissemination of pathogens by vectors possibly attracted to treated sewage sludge, it is essential that the material be applied quickly via surface spreading, injection, or incorporation and before it becomes attractive to vectors (USEPA 2000).

Another vector attraction option concerns the addition of alkaline material. With this option the ultimate decomposition of readily degradable material is postponed until the pH falls back to 10 or below, at which point biological decomposition resumes and odors can be produced and vectors attracted. In developing the lime stabilization process, it was intended that the treated sewage sludge be incorporated into the soil before the pH fell. Thorough mixing of lime with the sewage sludge and maintaining a high pH (> 10) are essential for vector and odor control. Reducing the moisture level of sewage sludge is another option for meeting vector attraction reduction requirements. However, when unstabilized dried material is remoistened, not only can microbial growth occur, but ammonia and other undesirable chemical emissions may result if the material is piled or spread in a thick layer so that it can readily become anaerobic. In the case of composting, workshop participants noted that care must be taken to avoid situations that lead to recontamination of material that has met PFRP, e.g., by exposing it to fresh organic materials such as untreated sewage sludge or by blending with other organic residuals containing readily available carbon and nitrogen sources.

In the long run, good management practices and common sense play critical roles in the recycling of treated sewage sludge and manure. Where the Class A or Class B material ultimately ends up dictates the appropriate management practice. For example, if the only area that a treatment plant has available for land application of treated sewage sludge is in close proximity to residences, surface application of a Class B product may not be the optimal management practice. The workshop participants agreed that some of the public's concerns about negative impacts from treated sewage sludge often are related strongly to the generation of malodors at treatment plants and land application sites. Many of these concerns could possibly be alleviated if more attention were given to odor control at the WWTP as well as at the land application and/or any interim storage site.

Oversight and Monitoring

Several major reports have been issued in recent years (National Academy 1996 and 2002; Inspector General Report 2000 and 2002) regarding regulation of sewage sludge in the United States. Each basically concluded that while the science behind the Part 503 rule is sound, the effectiveness of the rule is compromised by a lack of oversight and monitoring. A key part of that "sound science" is reflected in the answer to the core question of this chapter: When operated as intended, existing treatment technologies are adequate to disinfect sewage sludge and/or animal manures from all known pathogens, including emerging and reemerging pathogens. The operative phrase in that answer — which the authors of this chapter based on the findings of the 2001 workshop — is "when operated as intended." With Class B materials, "operated as intended" includes the critical time periods allowed for natural attenuation.

> Environmental Management Systems provide assurance to operators, regulators, and the public that the regulations are being properly implemented.

Experience with Class A and Class B sewage sludges over the past 12 years (since Part 503 was promulgated) — across a wide range of climatic zones with a variety of treatment systems — appears to show that treatment technologies and pathogenic/indicator microorganism tests on sewage

sludge are useful tools by which to gauge the efficiency and efficacy of treatments practices designed to minimize the hazard of public exposure to pathogens. The consensus of the microbe workgroups and risk assessment specialists who participated in the workshop was that data are still needed to document the actual field experiences, including the extent and effectiveness of field management practices, and to use that data to develop risk assessment models. The absence of clinically-documented cases of infections traceable to land application of treated sewage sludge does little to calm public apprehensions and perceptions of infection risk even when compared to situations involving obviously less treated materials like animal manure. Thus, the scientific community involved in this workshop (and subsequently the 2002 National Research Council Report, and the 2003 Biosolids Summit report (Dixon 2004)) recognized the need to work with the wastewater/sewage sludge and animal production industries to identify and prioritize rational research needs that can help with oversight and monitoring. This would be achieved primarily through the following steps: 1) Development of rapid, cost-effective, validated methods; 2) Bioaerosol and odor emission and transport studies; and 3) Incident tracking and evaluation relative to current practices.

Increasingly, it is being recognized that even when the federal and state requirements for sewage sludge disinfection and vector attraction reduction can be met, a publicly unacceptable (i.e., odorous, unsightly, etc.) product can still be generated. For example, if "Class A" compost is not adequately cured, it can be quite odorous. An alkaline-treated material can smell and look terrible if off gassing is incomplete, an odor-prone polymer was used in dewatering, or too much alkaline product was added and mixing was poor. Frequently, it is the nuisance factors caused by these odorous or unsightly products that attract the attention of the impacted public. Typically, it is when these "nuisance signals" are ignored or overlooked by utilities or their contractors, that the public is most negatively impacted. That is why proper management of sewage sludge from the moment of its production to the moment of its application and/or incorporation into the soil is essential to comply with regulatory requirements *and* pass the test of public scrutiny and tolerance.

Conclusion of National Academy Report, EPA Response

Several years ago, the USEPA commissioned a panel of the National Research Council (NRC) —part of the National Academy of Science — to review the technical basis of the regulations governing land application of treated sewage sludge. The panel had the opportunity to review the formal papers and workgroup discussions found in this publication. The NRC report, "Biosolids Applied to Land: Advancing Standards and Practices," was issued in July, 2002. The report noted that EPA does not have an adequate program to ensure compliance with the sewage sludge regulations and has not documented the effectiveness of its prescribed management practices. It also cited the need for further research on the public health aspects of sewage sludge recycling, stating in part: "There is no documented scientific evidence that the Part 503 rule has failed to protect public health. However, additional scientific work is needed to reduce the persistent uncertainty about the potential for adverse human health effects from exposure to treated sewage sludge. " It recommended that EPA expand its sewage sludge oversight activities to include procedures for:

1) Assessing the reliability of the sewage sludge treatment processes;

2) Monitoring compliance with the chemical and pathogen standards;

3) Conducting environmental hazard surveillance; and

4) Studying human exposure and health impacts.

EPA responded to the NRC report (EPA *Federal Register* response 2003) by proposing a number of research and data gathering projects. After receiving public comments to its proposal, EPA announced its final action plan on December 31, 2003, in a *Federal Register* notice. The plan includes a list of 14 projects the agency expects to complete or begin within the next two to three years, "with the goal of strengthening the sewage sludge use and disposal program." One project related directly to microbial pollutants is "Methods Development, Optimization, and Validation for Microbial Pollutants in Sewage Sludge." Four areas covered in this project are:

- Optimization of the method for detecting, enumerating, and determining the viability of *Ascaris* ova in sewage sludge;

- Improved methods for detecting viruses in sewage sludge;

- Development and validation of analytical methods for fecal coliform in sewage sludge;
- Development and validation of analytical methods for *Salmonella sp* in sewage sludge.

Other projects include:

1) Field Studies of Application of Treated Biosolids, especially measurement of bioaerosol and odor generation and transport, as well as natural attenuation of surface applied materials;
2) Participation in an Incident Tracking Workshop;
3) Conduct Exposure Measurement Workshop;
4) Assess the Quality and Utility of Data, Tools, and Methodologies to Conduct Microbial Risk Assessments on Pathogens;
5) Support Pathogen Equivalency Committee;
6) Development and Application of Analytical Methods for Detecting Pharmaceutical and Personal Care Products in Biosolids;
7) Improve Stakeholder Involvement and Risk Communication.

Program Oversight and Monitoring

The outcomes of these research and field studies will help to significantly expand the body of knowledge regarding beneficial use of treated sewage sludge and animal manure. In the meantime, operating treatment technologies "as intended" (along with best management practices) and instituting effective oversight and monitoring programs are critically important. State and federal regulators and other individuals overseeing sewage sludge programs have to assure that municipal wastewater treatment plants submit needed and additional data and/or employ different practices to assure that the public and environment are protected. (Regulators of CAFOs should create similar monitoring and oversight mechanisms.) Science has shown that treated sewage sludge can be safely used in many ways, but with little or no oversight, communities cannot be assured that appropriate practices are being implemented.

Some states have put in place additional protective practices, e.g. requiring six months of storage of Class B treated sewage sludge prior to application. This practice can be expected to further reduce the levels of any microorganisms present as well as any remaining biodegradable organic material. Other states limit the methods of application to incorporation and injection. To pay for staffing oversight programs, some states have instituted a per ton sewage sludge fee. States utilizing the methods mentioned above have had very few complaints about land application practices.

In all likelihood, however, decreasing federal and state budgets for environmental protection will make it increasingly difficult for regulators to provide close oversight of sewage sludge management practices. The Environmental Management System (EMS) program for biosolids will become an increasingly important tool for treatment plants that rely on land application of treated sewage sludge (or any other sewage sludge management option) to establish oversight and monitoring. An EMS can be used to establish a set of standard procedures that sewage sludge producers and appliers use to improve the effectiveness of their operations — both to meet regulatory requirements and address other issues of concern to communities like odor, noise, etc. Third party audits are required as a part of an EMS; such audits for small facilities with few resources will need to be conducted with the help of groups like the Rural Water Association who might also provide routine oversight with its circuit riders for both sewage sludge and large manure production operations. Wastewater treatment plants can adopt EMS concepts whether or not they choose to participate in the formal National Biosolids Partnership program.

The use of intermediaries and contractors for sewage sludge management, combined with ultimate disposition/recycling occurring at greater distances from the treatment plant, puts wastewater agencies in a position where it is more difficult for communities and citizens to communicate and build working relationships with them and to hold them accountable for quality of services. This situation contributes to public outrage when excessive odors, truck traffic, and other negative impacts occur.

Recent research on public perceptions of sewage sludge recycling (Beecher et al. 2004) found that strengthening public relationships — especially allowing for two-way communications — is a valuable

tool in building sustainability into a "long distance" (or any type of) management program, for which the sludge preparer is ultimately responsible even when a contract applier is retained. Both traditional stakeholders (regulators, plant managers, land applicators, etc.) and nontraditional stakeholders (concerned members of the public) need to be kept regularly apprised of how residuals are being treated and beneficially used. Frequent and open communication between all stakeholders is critical in any treated sewage sludge recycling program (and is especially useful when public uncertainty exists). These communications provide an opportunity to describe the way site monitoring and access restrictions will be achieved and enable the industry and the community to quickly respond to critical concerns such as annoyances related to odors and perceived or actual health effects. In addition, engineers, scientists, and technicians must learn to communicate in understandable terms with the public and earn their trust. Open and two-way communication also will facilitate dealing with issues for which definitive scientific answers are not available at the present time. The ultimate outcome of establishing productive public relationships is a high quality operation that withstands public scrutiny.

Conclusions

In terms of infectious disease agents in sewage sludge and manure, what is presently known is that for disease to occur, the host (human or animal) must come into contact with the infectious agent (pathogen). Hence, the first line of defense is to disinfect the material. Current treatment technology options for Class A sewage sludge, when designed and operated in accord with 40CFR503, reduce the pathogen load to below the detection limit. The second line of defense involves treating material in such a way that it no longer attracts vectors that might disseminate disease agents away from and/or to the storage or application site, or allows growth of undetectable or reintroduced bacterial pathogens. This involves achieving a level of vector attraction reduction in accord with 40CFR503. Together these two conditions — disinfection and vector attraction reduction — are protective of public health. Class B treatment technologies, combined with field treatment (i.e., natural attenuation) to reduce pathogen levels below the detection point, also have been shown to be maximally protective of public health.

Pathogen viability and infectiousness are the endpoints that can truly impact public or animal health and the environment. Detection alone by one of the molecular methods, without evidence of infectiousness, leaves interpretation of test results unresolved (i.e., the test identifies whether a disease agent is or was present, but does not identify the source or whether the agent is viable). Ideally, perhaps newer methods will address both viability and infectiousness. While better methods are needed for emerging pathogens, equally important is the standardization and validation of methods for the microorganisms currently specified in the 40CFR503 regulation — fecal coliforms, *Salmonella sp.*, enteric viruses, and helminth ova. Methods for validation of the fecal coliform and *Salmonella sp.* procedures for sewage sludge are underway in 2004.

A concern — addressed in Part I in the response to the question about better detection methods — is finding larger number of organisms in a gram of sewage sludge with new methodologies than with older ones. What does that mean? The absolute risk is the same, but will appear greater and perhaps become a concern to the public. It is likely that a risk assessment will be needed to show that there is no change in the risk because of a change in the analytical method.

The public wants an indicator of pathogen risk and safety; yet the best science can do at the present and for the foreseeable future is determine if the soil, water, crops, or air are contaminated by some animal or human fecal material. As noted in the introduction to this chapter, there is an absence of documented food and water-borne illness outbreaks related to treated sewage sludge in the U.S. In contrast, documented illness outbreaks related to animal manure have been extensively reported (Beuchat 1996; Tauxe 2002) and hence there is a need for examination of treatment technologies available for animal manures (Bicudo and Goyal 2003). The main vulnerability to human health is the lack of oversight and monitoring that ensures treatment technologies are being operated as designed, and that access restrictions are suitable and implemented. Growing pressure on the part of the public, along with adoption of Environmental Management Systems, are expected to improve oversight and monitoring of treated sewage sludge management programs. Increasingly, livestock operations also are employing tools, e.g., composting, wastewater treatment, and biomass-to-energy conversions, which minimize the public's potential exposure to infectious disease agents.

The current interest in and support for microbial risk assessments has been expanding (Gale 2001; Haas 2002; Westrell et al. 2004). Progress in the field of microbiological risk assessment relative to foodborne illness pathogens (Lammerding and Fazil, 2000) can be expected to contribute substantially to advances in microbial risk assessments for sewage sludge, manure, and other residuals.

In summary, workshop participants generally agreed on the following points:

- There are currently available treatment technologies that *per se* adequately disinfect sewage sludge of existing and emerging pathogenic microorganisms to a degree that has consistently resulted in no clinically-documented outbreaks of illness that were associated with land application of treated sewage sludge.

- There are several currently available treatment technologies per se that can adequately disinfect animal manure of existing and emerging pathogenic microorganisms. However, many of these technologies are still either underutilized or inadequately implemented with regard to the goal of pathogen disinfection. In addition, previous general absence of appropriate guidance and use of microbial-hazard prevention practices for land application of animal manure resulted in several clinically-documented illness outbreaks associated with fecal contamination from animal manure.

- Release and transport of odor and volatile compounds during land application of raw or treated animal manure and treated sewage sludge are realities that need to be addressed via good management practices. These emissions have the potential for public annoyance, eye, nose, and throat irritation, and feeling of sickness and can occur even when the presence of pathogens is undetectable in highly treated materials.

- Analytical methods for detecting and quantifying existing pathogens and indicators must be standardized and validated, and methods for detecting and quantifying emerging pathogens in sewage sludge and animal manures need to be developed, standardized, and validated.

- Pathogen quantification results (using modern validated methods) along with determination of the viability/infectious capacity of pathogens need to be considered together and in a microbial risk assessment and infection outcome context.

- The current sewage sludge and animal manure treatment technologies can be expected to affect antibiotic resistant as well as nonresistant strains of bacteria equally. Furthermore, after land application the inherent capacity for these bacteria to maintain antibiotic resistance can be expected to diminish as the concentrations of the corresponding antibiotics decrease in the soil environment. More evidence is needed to understand to what extent the genetic elements carrying the antibiotic resistant traits actually, rather than just theoretically, are surviving and possibly increasing in the environment.

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Why a Workshop on Emerging Infectious Disease Agents and Issues Associated with Animal Manures, Biosolids and Other Similar By-Products?

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Issues

Each year in the United States, 7 million dry tons of municipal wastewater solids and almost 10 times as much animal wastes are produced (WEF 2001). A large percentage (about 60%) of these materials is applied to land for use in agriculture (Smith & Brobst 2001). The land application of municipal biosolids is regulated by the U.S. Environmental Protection Agency (USEPA 1993). Conversely, land application of animal wastes is little regulated at the federal level. (EPA adopted regulations for Confined Animal Feeding Operations (CAFOs), which forbid the direct/indirect discharge of wastes to surface waters and require agronomic application rates for manure used on CAFO owned property. The regulation does not directly address pathogens, although it is thought — by requiring agronomic application rates — some measure of reduction will result). Activities like land application in watersheds can be expected to grow as our populations increase. From a watershed perspective, the application of municipal biosolids and animal wastes can mean large quantities of potentially pathogenic microorganisms are being applied to land and may find their way into water supplies, recreational waters and food (animals and crops). In addition to these direct routes of exposure, contact with human, animal, or insect carriers are other potential routes of exposure of the population. An awareness of these possibilities raises numerous public health concerns. A number of waterborne disease outbreaks are listed in Table 1 (Rosen 2000), together with the causative organism and whether the contaminated waterway source was a surface or groundwater supply. While none of these outbreaks were directly associated with the use of manure or biosolids, note that the organisms identified are not strangers and can be expected to be present in untreated sludges and manures. These organisms cause a variety of diseases including gastrointestinal problems and more serious illnesses that may require hospitalization and even result in death. For example, *Cryptosporidium* and *Escherichia coli* O157:H7 have both resulted in mortalities after waterborne outbreaks affecting thousands of people in the U.S. and Canada (Valcour et al. 2002; Public Health Dispatch 1999). At the state fair in Albany, New York, manure contaminated ground water during heavy rains, and this water was used to prepare food, drinks and ice (Public Health Dispatch 1999). Food-borne outbreaks of disease, resulting from manure fertilization of crops that can be eaten raw, like lettuce, have also been reported (USEPA 1998).

Microorganisms in Sludge/Biosolids

During the course of typical wastewater treatment, the microorganisms in sewage are reduced in number, then are concentrated in the sewage sludge, the solids-ladened portion, and separated from the treated wastewater. As the sludge is further treated, and becomes a useful organic soil amendment known as "biosolids," the pathogen numbers decline further to a level that depends on the type of treatment and the treatment parameters such as time, temperature, pH, mixing, moisture, etc.

(Editor's Note: The U.S. EPA Part 503 regulations do not use the term "biosolids," only sewage sludge and treated sewage sludge. In this publication, the editors have chosen to use the word biosolids when appropriate. The word biosolids was introduced and defined by the Water Environment Federation and has been used in the profession since the early 1990s. Biosolids is defined as followed (see: http://www.engr.psu.edu/ce/enve/publications/biosolids.pdf):"Biosolids is a more precise term properly used only to describe that portion of the wastewater solids stream which meets federal and state regulations for beneficial use by land application or other methods.")

Table 1. Waterborne Disease Outbreaks causing Gastroenteritis 1989-1996

Type of Organism	Agent	Number of Outbreaks	Outbreaks Associated with Drinking Water		Outbreaks Associated with Recreational Water	
			Surface	Ground	Natural	Pool/Park
Protozoa	*Giardia* spp.	27	12	6	4	5
	Cryptosporidium parvum	21	4	4	2	11
Bacteria with Potential for Infecting Multiple Species	*Escherichia coli* O157:H7	11		3	7	1
	Campylobacter jejuni					
	Salmonella typhimurium	3 1	3	1		
	Salmonella java	1				1
	Leptospira grippotyphosa	1			1	
Bacterial Infections Associated with Humans	*Shigella sonnei*	17		7	10	
	Shigella flexneri	2		1	1	
Human Viruses	Hepatitus A	3				3
	Norwalk virus	1		1		
	Norwalk like virus	1				1
	Small round structured virus	1	1			
Acute Gastroenteritis	Unidentified cause–many consistent with viral epidemiology	60	8	44	7	1
Other	Cyanobacteria-like bodies	1	1			

Table 2 shows some pathogens of concern in municipal wastewater and sewage sludge and associated diseases/symptoms that they may produce (USEPA 1985 1989). We need to ask ourselves: Is this list complete? Are there emerging organisms of concern that should be addressed?

Microorganisms in Animal Wastes

In the United States, about 1.3 billion tons of manure are produced annually and much of it is used in agriculture (Anon 1998). Table 3 shows some pathogenic microorganisms that may be present in animal manures. Residuals from animal raising operations are valuable resources for agriculture because of their nitrogen, phosphorus, potassium, organic material, and trace nutrient content. Unfortunately they can also contain undesirable constituents like organisms that are pathogenic to humans and/or animals. In recent years several water contamination incidents and several outbreaks of food borne illnesses have been associated with domestic animal feces or animal manures. Although there are numerous human pathogenic organisms found in manures (see Table 3 in Smith & Epstein 1999), relatively few organisms have been linked

Table 2. Some Pathogens of Concern in Municipal Wastewater and Sewage Sludge

Bacteria	Disease/Symptoms for Organism
Salmonella spp.	Salmonellosis (food poisoning), typhoid
Shigella spp.	Bacillary dysentery
Yersinia spp.	Acute gastroenteritis (diarrhea, abdominal pain)
Vibrio cholerae	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli (pathogenic strains)	Gastroenteritis
Viruses	
Poliovirus	Poliomyelitis
Coxsackievirus	Meningitis, pneumonia, hepatitis, fever, etc.
Echovirus	Meningitis, paralysis, encephalitis, fever, etc.
Hepatitis A virus	Infectious hepatitis
Rotavirus	Acute gastroenteritis with severe diarrhea
Norwalk Agents	Epidemic gastroenteritis with severe diarrhea
Reovirus	Respiratory infections, gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis, Cryptosporidiosis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (diarrhea & abdominal cramps)
Balantidium coli	Diarrhea and dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth Worms	
Ascaris lumbricoides	Digestive disturbances, abdominal pain
Ascaris suum	Can have symptoms: coughing, chest pain
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort & muscle aches
Taenia saginata	Nervousness, insomnia, anorexia
Taenia solium	Nervousness, insomnia, anorexia
Necator americanus	Hookworm disease
Hymenolepis nana	Taeniasis

to outbreaks associated with the use of manure. The predominant ones are *Salmonella* spp., *Escherichia coli* O157:H7, *Cryptosporidium parvum* and *Listeria monocytogenes* (Valcour et al. 2002; Public Health Dispatch 1999; USEPA 1998). Again we must ask: Is this list complete? Are all the important organisms noted? Are there newly emerging ones that should be included?

Detection and Identification of Pathogenic Microorganisms

It is important that the pathogenic microorganisms (both human and animal) potentially present in municipal biosolids and animal wastes be identified, and the risks they pose to human health be assessed and characterized.

Table 3. Some Microorganisms in Animal Manures Which Can Cause Disease to Humans

Bacteria	Disease
Campylobacter jejuni	Bloody diarrhea, abdominal pain
Escherichia coli	Gastrointestinal disease
Leptospira spp.	Kidney infection
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium paratuberculosis	Johnes disease
Yersinia enterocolitica	Gastrointestinal infection
Viruses	
Adenoviruses	Eye and respiratory infections
Avian enteroviruses	Respiratory infections
Avian reoviruses	Infectious bronchitis
Bovine parvovirus	Respiratory infections
Bovine rhinovirus	Foot and mouth disease
Enterovirus	Respiratory infections
Reovirus	Respiratory infections
Rhinovirus	Parainfluenza
Rotaviruses	Gastrointestinal infections
Parasites - Protozoa	
Balantidium coli	Balantidiasis
Cryptosporidium parvum	Cryptosporidiosis
Eimeria spp.	Coccidiosis
Giardia lamblia	Giardiasis
Toxoplasma spp.	Toxoplasmosis
Parasites - Helminths	
Ascaris lumbricoides	Ascariasis

Because of the difficulty, time and expense associated with analyzing environmental samples for pathogens, indicator microorganisms have been used to signify their potential presence. The most common indicators are bacteria that live in the enteric tracts of humans and other animals and for which testing is relatively rapid, inexpensive, and easy. These include total coliforms, fecal coliforms, *E. coli* and Enterococcus spp. With sludges and animal wastes, fecal coliforms are typically employed to indicate the potential presence of pathogens. We must ask, Are they the best indicator? Can their presence or absence be correlated with the presence or absence of pathogenic bacteria, viruses, and/or parasites? Is there a better indicator for this purpose? What does it mean to find low levels of fecal coliforms? What does it mean to find no enteric viruses or helminth ova in sludge? What are comparable indicator organisms to be considering in animal wastes?

A second use of indicator organisms is as a measure of treatment efficiency. For example, fecal coliform density following treatment is employed as a measure of how well a process can meet Class B sewage sludge disinfection requirements, it being a basis for many of the so called Processes to Significantly Reduce Pathogens (PSRP) requirements. (*Editor's Note: See paper by J.B. Farrell, these proceedings, for a detailed discussion of Class A and Class B requirements under the 1993 40 CFR Part 503 regulations.)* For Class A and the Processes to Further Reduce Pathogens (PFRP) requirements, the ability to reduce enteric viruses and helminth ova in sewage sludge to below detectable levels was employed as a measure of treatment effectiveness. Is this the best approach to determine disinfection capability of a process? Is it correct to assume that if enteric viruses and helminth ova are reduced all pathogens will be reduced?

Infectious Dose

While pathogenic organisms are present in the natural environment, animal wastes and sewage/wastewater, and they may accumulate or concentrate in the sewage sludge, a key question to answer for conducting risk assessments is: What constitutes an infectious dose for a pathogenic organism? The infectious dose varies with the organism and, particularly for bacteria and viruses, can vary widely. The infectious dose can even vary for the same species of microorganism. For example, recent studies in volunteers have shown that *Cryptosporidium parvum* can range in infectious dose from 10^1 to 10^3 oocysts (Okhuysen et al 1999). Table 4 gives some reported infective dose data, showing values as low as 100 for *Salmonella* (various species), < 1 PFU for poliovirus, and 1 viable egg for helminths. Are these values still accurate? Since the data presented are generally for healthy adults, the numbers for young, elderly, and immunocompromised individuals may be lower. Is new information surfacing as we continue to investigate microbiological risk assessments?

Reducing the Risk of Infection

Survival times of pathogens on soil and plants can vary from days to years (Kowal 1985). Generally, helminths may be expected to survive the longest, followed by bacteria and viruses; the protozoan cysts have the shortest survival times. Since the survival times are variable and can be lengthy, before sewage sludge is applied to land, it should have its vector attractiveness reduced and be either totally disinfected or partially disinfected and applied under restrictions that prevent it from being a risk to public health and the environment. If the treated sludge (biosolids) is to be used without restrictions (Class A), i.e., with crops that may be eaten raw, come into contact with the public, or marketed, it must be treated in such a way

Table 4. Reported Infective Dose Data

Organism	Infective Dose	Range	Reference
Bacteria			
Clostridium perfringens	10^6	10^6 to 10^{10}	Kowal, 1985
Escherichia coli	10^4	10^4 to 10^{10}	Kowal, 1985; Keswick, 1984
Salmonella (various species)	10^2	10^2 to 10^{10}	Kowal, 1985
Shigella dysenteriae	10 to 10^2	10 to 10^9	Kowal, 1985; Keswick, 1984; Levine, 1973
Shigella flexneri	10^2	10^2 to 10^9	Kowal, 1985
Streptococcus faecalis	10^9	10^9 to 10^{10}	Kowal, 1985
Vibrio cholerae	10^3	10^3 to 10^{11}	Kowal, 1985; Keswick, 1984
Viruses			
Echovirus 12	HID50 919 PFU	17-919 PFU	Kowal, 1985
	HID1 17 PFU est'd		
Poliovirus	1 TCID50, <1 PFU	4 x 10^7 TCID50 for infants	Kowal, 1985
		0.2 to 5.5 x 10^6 PFU for infants	
Rotavirus	HID50 10 ffu	0.9 to 9 x 10^4 ffu	Ward et al., 1986
	HID25 1 ffu est'd		
Parasites			
Entamoeba coli	1-10 cysts	1-10 cysts	Kowal, 1985
Cryptosporidium	10 cysts	10-100 cysts	Casemore, 1991
Giardia lamblia	1 cyst estimated	NR	Kowal, 1985
Helminths	1 egg	NR	Kowal, 1985

HID = human infective dose; TCID50 = tissue culture infectious dose for 50 % response; PFU = plaque forming units; ffu = focus forming units; NR = not reported

that pathogenic microorganisms are reduced below detection limits. Pathogens of concern, and those also used as indicators of other pathogens in Class A biosolids, are *Salmonella* sp., enteroviruses, and *Ascaris* sp. Processes employed for achieving this level of treatment usually involve holding the sludge at a temperature between 50° and 85°C for a period of time. Other sludges used in agriculture must receive a minimal level of treatment (Class B) which means treatment by anaerobic digestion, aerobic digestion or lime treatment to significantly reduce the level of pathogens. The fecal coliforms are reduced by two $\log_{10}$ and the *Salmonella* sp. are estimated to be reduced by one $\log_{10}$. However, some pathogenic organisms may still be present. As such, precautionary measures must be exercised to insure that the risk to public health is minimized. These measures include restricting the public's access to the land application site, controlling animal grazing, and preventing crop harvesting for various periods of time depending on the crop and method of biosolids application.

The land application of animal wastes is little if at all regulated. (EPA adopted regulations for Confined Animal Feeding Operations (CAFOS), which require agronomic application rates for manure only on land owned by the CAFO. The regulation does not address pathogens.) Treatment methods, such as waste stabilization ponds, storage, and composting, are applied by some operations for the purpose of stabilizing the wastes and reducing contaminants including pathogens.

Expectations of Conference

A group of national and international technical specialists in environmental microbiology and the control of pathogenic microorganisms gathered to participate in this workshop. The goal of their efforts is to produce an assessment of the state of the science associated with the occurrence, detection, and destruction of infectious disease agents in sewage sludge/biosolids, manure, and other waste processing by-products that are usually treated and subsequently beneficially utilized.

Following presentations by invited speakers on pathogenic bacteria, viruses, parasites, control technology and risk assessment, workshop participants were divided into discussion groups to review, evaluate and update the existing information and data. Among the questions that the discussion groups addressed are:

- What is known about the fate and transport — including detection, enumeration, viability, survival, destruction, transmission, and dissemination pathways — of these disease agents?
- What is known about the treatment technologies in terms of theoretical and actual effectiveness?
- What is known about current utilization and handling practices relative to pathogenic agent survival?
- Is risk assessment and cost-benefit information/data available for presentation and discussion?

It is expected that the workshop proceedings will be used by those with a need to determine the state of knowledge on the detection, occurrence, health significance and control of infectious disease agents in sewage sludges, manures and other wastes.

In summary, a number of pathogens may be found in municipal sewage sludges and animal wastes. To address concerns about the potential for transmission of these pathogens to humans and animals, a group of international experts assembled to examine the adequacy of existing treatment technologies and their associated utilization practices to reduce, destroy, or inactivate the spectrum of infectious agents currently or potentially present in biosolids, manure, or other by-products.

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SECTION II

Bacteria in Biosolids/Treated Sewage Sludge and Animal Waste

Bacterial Pathogens in Biosolids – Emerging Issues

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Introduction

A number of federal agencies and academic foundations cosponsored workshops in 1973 and again in 1983 to examine issues and identify research needs to assure safe recycling of municipal sludges and effluents on land (Page et al. 1983). These workshops provided the stimulus for an extensive program of research conducted by federal, state, municipal and private agencies. That research subsequently provided data used for the promulgation of the 1993 40 CFR Part 503 regulations (USEPA 1993). Since then, land application of biosolids has grown steadily, but not without problems. Public opposition to land application programs has also grown in many areas of the country, especially with the use of specific Class B products as defined in the 503 regulations. Odors often trigger the initial expressions of public concern, which in turn lead to questions about the fate and potential risks associated with chemical and biological agents potentially present in biosolids.*(Editor's Note: See paper by J.B. Farrell, these proceedings, for a discussion of Class A and Class B requirements under the 1993 40 CFR Part 503 regulations.)*

Public awareness and concern about infectious diseases in general have been increasing in the U.S. since the adoption of the 503 regulations. The public is regularly exposed to headlines about AIDS, flesh-eating bacteria, "killer hamburgers", Hanta virus, Ebola virus, *Cryptosporidium* in water and antibiotic resistance, just to name a few. While these all represent real public health issues, the popular press does not always provide appropriate perspective, and fictionalized versions in popular novels and movies often blur reality. At the same time, rates of infectious disease have been increasing in the U.S. and it was widely reported that between 1980 and 1992 infectious diseases "leaped over" accidents and strokes to now stand in third place behind heart disease and cancer as leading causes of death (Garrett 1994). While that may be alarming, it would also appear to be somewhat misleading. The increased death rate from infectious diseases was significantly impacted by the AIDS epidemic at the same time that deaths due to accidents and strokes were decreasing (Murray 2001). The decrease in death rates from accidents and strokes is good news reflecting increased emphasis on safety and improved treatments and medications.

It nevertheless remains true that some infectious disease rates have been increasing, and new and emerging pathogens are appearing. To that extent, increased public concern is appropriate and warranted. Many of the causes for the increases in infectious disease are known or can be reasonably hypothesized. While there is no evidence that land application of treated biosolids has contributed to the increase in infectious disease rates, public concerns about land application cannot be ignored. Unfortunately, there is little data available about the fate of emerging pathogens in biosolids treatment processes and land application programs. Research on the fate of microorganisms in biosolids essentially stopped in the U.S. after the adoption of the 503 regulations.

This paper will review potential issues specifically related to bacterial pathogens by focusing on earlier reviews by Gerba and Kowal that were presented at the 1983 Denver workshop (Page et al. 1983) and a 1987 national "occurrence" study conducted for USEPA by Yanko (1987). Workshop and research findings and recommendations will be reviewed in context of their relevance today. The role of laboratory methods and the impact of testing methods on the reliability of available research data and the effectiveness of process monitoring will also be discussed.

Utilization of Municipal Wastewater and Sludge on Land, 1983 Denver Workshop

The 1983 Denver workshop was sponsored by USEPA, the U.S. Army Corps of Engineers, USDA Cooperative State Research Service, National Science Foundation and University of California-Kearney Foundation of Soil Science. The workshop incorporated a broad range of topics including political and institutional contraints, engineering systems, management considerations and public health and risk assessment for both pathogens and chemical constituents. Gerba (in Page et al. 1987) presented a detailed review of the occurrence of pathogens in wastewater, including bacteria, parasites and viruses, and their fate in treatment processes and the environment. Kowal (in Page et al. 1987) added to that material with an extensive review of the potential health effects associated with both microbial and chemical contaminants.Gerba and Kowal both identified a list of bacterial pathogens that were potentially a major concern with land application programs. Kowal expanded on that with a second list of bacterial pathogens that were considered to be of minor concern for land application. Table 1 summarizes the 1983 listings of bacterial pathogens and suggests some changes and new issues to consider that were not included 18 years ago.

The bacterial pathogens that were categorized as of major concern in Table 1 are sewage-borne pathogens that have significant disease impacts on a worldwide basis. Some on that list cause relatively uncommon diseases in the U.S. *Salmonella* and *Shigella* cause the most common reported enteric bacterial infections in the U.S., however, an active surveillance program indicated that the incidence of *Campylobacter* infections exceeded that of *Salmonella* and *Shigella* (MMWR 1999). Incidence rates for the common foodborne bacterial diseases are shown in Table 2 (MMWR 1999). While the true incidence rates for these diseases due to all causes are not known, most *Campylobacter* and *Salmonella* infections in the U.S. are foodborne. There is no known association of biosolids with foodborne outbreaks of the diseases listed in Table 2. Contamination of implicated foods is most likely through some contact with manure, either directly or indirectly through irrigation or process water. Since millions of cases of these diseases occur in the U.S. each year, the microorganisms are likely to be present in the raw sewage of municipal treatment plants. Most research on the fate of bacterial pathogens has been based on the use of indicator bacteria, such as fecal coliform, or direct measurement of *Salmonella*. Although *Campylobacter* is now recognized as being the cause of the most common enteric bacterial infection, there is relatively little research in the U.S. documenting its fate in biosolids treatment processes or in the environment. The relative lack of data about the fate of *Campylobacter* is most likely due to three factors: 1) the high incidence of *Campylobacter* infections was previously not fully appreciated; 2) the available laboratory methods were not adequate to reliably detect *Campylobacter* in biosolids; and 3) conventional wisdom suggested this organism was more fastidious and probably more easily

Table 1. Bacterial Pathogens of Potential Concern in Biosolids

Bacterial Pathogens 1983 Workshop (Page et al., 1987)		**2001**
Major Concern (Gerba & Kowal)	**Minor Concern (Kowal)**	**Potential Changes and New Issues**
Salmonella spp. *Shigella* spp. *enterpathogenic E. coli* *Yersinia enterocolitica* *Campylobacter jejuni* *Vibrio cholera* *Leptospira* spp.	*Aeromonas* spp *Bacillus cereus* *Brucella* spp. *Citrobacter* spp. *Clostridium perfringens* *Coxiella burnetii* *Enterobacter* spp. *Erysipelotrix rhusiopathiae* *Francisella tularensis* *Klebsiella* spp. *Legionella pneumophilia* *Listeria monocytogenes* *Mycobacterium tuberculosis* *Mycobacterium* spp. *Proteus* spp. *Pseudomonas aeruginosa* *Serratia* spp. *Staphylococcus* spp. *Streptococcus* spp.	*E. coli* 0157:H7 *Listeria* spp. *Mycobacteria* spp. *Helicobacter* spp. *Aeromonas* spp. *Burkholderia* spp. *Legionella* spp. Endotoxins Antibiotic resistance

Table 2. Rate per 100,000 population of selected pathogens detected by the Foodborne Diseases Active Surveillance Network (Foodnet) at the five original sites, by year—United States, 1996-1998

Organism	1996	1997	1998
Campylobacter	23.5	25.2	21.7
Salmonella	14.5	13.6	12.4
Shigella	8.9	7.5	8.5
E. coli 0157:H7	2.7	2.3	2.8
Yersinia	1.0	0.9	1.0
Listeria	0.5	0.5	0.5
Vibrio	0.1	0.3	0.3
Total	51.2	50.3	47.2

inactivated than the indicator bacteria or *Salmonella*. The high incidence of foodborne *Campylobacter* infections suggests that last assumption may not be entirely accurate. Researchers in the UK have reported that mesophilic anaerobic digestion had little effect in reducing the viable numbers of *Campylobacter* (Kearney et al 1993). Thermal inactivation of *Campylobacter* starts at about 48°C, so high temperature Class A processes would be expected to destroy *Campylobacter*.

The *Vibrio* incidence reported in Table 2 represents predominantly *Vibrio parahaemolyticus* infections. Cholera is rare in industrialized countries. While this disease remains pandemic in some parts of the world and outbreaks continue to occur in underdeveloped countries, there are only 0–5 cases of cholera per year documented in the U.S. (CDC Online 2001a). Leptospirosis also has a very low known incidence rate in the U.S., with 100–200 documented cases per year, half occurring in Hawaii (CDC Online 2001b). Both *Vibrio cholera* and *Leptospira* were listed in Table 1 as diseases of major concern associated with sewage. There is little information about the fate of these organisms in biosolids treatment processes or land application programs, however, the extremely low incidence of these diseases in the U.S. justifies the decision to focus research resources in other areas.

Yersinia enterocolitica has become recognized worldwide as an important human pathogen and has been reported to be as common as *Salmonella* and *Campylobacter* in some countries (Fenwick 1995). While infection rates remain relatively low in the U.S., as noted in Table 2, the serotype most often isolated in the U.S. has been changing and is starting to reflect the serotype most often detected in clinical samples in other parts of the world where this pathogen is more common (Bissett 1990). The implications of this shift are not yet apparent, but may reflect the increase and ease of world travel and the worldwide distribution of food products. The fate of *Yersinia* in biosolids will be discussed in greater detail later in this paper.

Table 1 also listed enteropathogenic *E. coli* as a potential major concern. This generic group of pathogenic *E. coli* was recognized as having potential health implications, but there was little data at the time of the 1983 Denver workshop suggesting any significant health risk in the U.S. *E coli* 0157:H7, a particularly virulent strain of toxigenic *E. coli*, was first reported in 1982 and thus was not recognized as a potential risk at the 1983 workshop. It is now well documented that *E. coli* 0157:H7 is a significant foodborne pathogen (Table 2) and can be transmitted via environmental routes, such as groundwater and recreational water. The true numbers of the larger group of toxigenic *E. coli* infections is still not known due to the difficulty of identifying these strains. The question of toxigenic *E. coli* in biosolids will also be explored later in this discussion.

The bacterial pathogens listed in the minor concern category in Table 1 were opportunistic pathogens that are widespread in the environment or pathogens that were thought unlikely to have a fecal-oral or biosolids-associated route of transmission. For the most part, that assessment still appears valid, but reconsideration of a few in that list may be warranted. The third column of Table 1 lists potential biosolids-related bacterial pathogen issues that were not evaluated or for which there is little data to make an assessment. Most of these organisms have been implicated as pathogens of concern in drinking water or food, and by inference, their fate in biosolids and land application programs should be considered. At the same time,

many of these organisms illustrate the difficulty of providing perspective and making a reasoned risk-based assessment. *Aeromonas* and *Mycobacterium avium* complex are ubiqiutous organisms in the environment. Concern about their presence in drinking water has surfaced predominantly due to greater numbers of an immunocompromised or susceptible population. It may be viewed as an undesirable outcome if a land application program significantly increased their numbers in a particular environment. On the other hand, the mere presence of these organisms in biosolids at concentrations typically found in the environment would not impact inherent environmental risks for susceptible individuals.

Legionella presents a particularly interesting question to consider. *Legionella* is another organism that is ubiquitous in the environment. It is typically found in surface waters at concentrations of 10^4-10^5 per liter (Fliermans et al. 1981) and is now recognized as part of the natural aquatic environment. Outbreaks of Legionnaires' Disease are typically associated with airborne transmission of *Legionella* bacteria through cooling towers, showers and other aerosolizing devices. Infections with one species, *Legionella longbeachae*, have been associated with gardening and the use of potting soil in Australia and Japan. At least three cases of Legionnaires' Disease in the U.S. have been associated with potting soil (MMWR 2000). Given the propensity for *Legionella* to grow in hot water, such as cooling towers or hot water distribution systems and the ubiquitous distribution of this organism, it is reasonable to hypothesize that it may also grow well in warm, self-composting organic masses. That growth could increase organism densities to levels that represent a potential risk from aerosol transmission to susceptible individuals. If this scenario indeed represents a risk, it would appear to be a theoretical risk associated with any self-composting organic mass, and not limited to sewage-derived biosolids. Class A controlled composting or heat treatment, as defined in the 503 regulations, would be expected to kill *Legionella longbeachae.* Steele et al (1990) indicated that a temperature of 43°C in soil was lethal to this species, suggesting it would not be an issue in Class A biosolids products. Nothing is known about the potential for *Legionella* to repopulate in warm biosolids stockpiles following the controlled elevated temperature process. While the above discussion suggests some concern may be warranted, that concern must be tempered by the fact that there is no known case of Legionnaires' Disease associated with either the production or land application of biosolids. There is, however, some evidence that workers at a food industry sewage treatment plant in Denmark contracted Pontiac Fever (nonpneumonic legionellosis) from aerosols emitted in the treatment plant (Gregersen et al. 1999).

Listeria is another bacterial pathogen that is common in sewage, but is also commonly found in soil and on vegetation, indicating its existence in nature as a saprophyte (Watkins and Sleath 1981). The organism is also found in the feces of wildlife and birds (Kampelmacher and van Noorle Jansen, 1980). It has been detected in both surface and groundwater, on store-bought food products and in the feces of healthy people. Both the hemolytic and non-hemolytic forms of *Listeria monocytogenes* have been isolated from all environments, including healthy people. A lower percentage of environmental strains are pathogenic compared to those isolated from humans and animals. It is questionable if the nonhemolytic form has any clinical significance. German researchers indicated that *Listeria* was not reduced by biological oxidation, and may actually increase in some cases and become concentrated in sludges (Geuenich and Muller 1984). Anaerobic digestion, however, reduces the numbers of Listeria (DeLuca et al. 1998). Given the widespread environmental distribution of *Listeria,* the infection rate remains relatively low. As with the other opportunistic pathogens, there is a strong relationship between predisposing factors and pathogenicity, with the fetuses of pregnant women at greatest risk (Farber and Losos 1988).

Helicobacter pylori has been found to be associated with gastritis and with gastric and duodenal ulcers (AWWARF Online 1997). Diseases of the stomach appear to be multifactorial and infection by *H. pylori* is probably one part of a sequence of events required to produce disease. *H. pylori* has emerged as an important risk factor for gastric cancer and is considered a Class I carcinogen by the International Agency for Research on Cancer (Friis *et al* 1996). Epidemiologic studies suggest *H. pylori* infection is possibly the most prevalent bacterial infection in man, however, only a small portion will develop any symptoms, and rates of infections have been falling. A mathematical model developed by researchers at Stanford suggested that in the United States transmissibility of *H. pylori* has decreased to values so low that, should the trend continue, the organism will disappear from the population without targeted intervention over the next century (Rupnow et al. 2000). Environmental sources of *H. pylori* remain unclear. The digestive tract of humans appears to be the main reservoir of *H. pylori* and sewage pollution is therefore a possible, but not proven, route of infection. One study in Chile suggested that uncooked vegetables irrigated with water contaminated by raw sewage may be a route of infection. Two studies of sewage workers, one in Singapore and one in Sweden, found no increased risk of infection from *H. pylori* from exposures in sewage work (Friis et al. 1996; Tai 2000). There are no data about the fate of *H. pylori* in biosolids treatment processes.

Burkholderia cepacia (previously classified as *Pseudomonas cepacia*) is a bacterium that is attracting much attention as a plant pathogen, saprophyte, biocontrol agent, bioremediation agent and human pathogen (Parke, 1998). This organism further illustrates the complexities of addressing potential health issues associated with land application of biosolids. *B. cepacia* is widely distributed in nature and naturally abundant in soil, water, sewage and on plant surfaces.

In the past 20 years *B. cepacia* has also emerged as a human pathogen, particularly among cystic fibrosis (CF) patients. It has been isolated as a contaminant of anesthetic solutions, humidifiers, contact lens solutions and hemodialysis machines (Hospital Epdemiology 2001). It can cause a wide variety of infections in immunocompromised hosts, but is of particular concern as a respiratory pathogen in CF patients where it significantly increases death rates (Holmes *et al*. 1998). The organism also has extraordinary metabolic versatility and can degrade chlorinated aromatic substrates (Holmes et al. 1998), an ability which has spurred development of *B. cepacia* for use in bioremediation of soil and groundwater contaminated with chlorinated hydrocarbons. Many strains also produce one or more antibiotics active against a broad range of plant pathogenic fungi. Much research has been devoted to developing the organism as a biological control agent to replace the use of chemical pesticides. While EPA is proceeding cautiously on approving permits for use as a biological control agent, at least one product (trade name Blue Circle) and associated application have been approved. Some in the medical community have expressed concern about potential uses of *B. cepacia* as a biocontrol agent, suggesting that the agricultural application of *B. cepacia* will lead to environmental and water contamination and increased human exposure (Holmes et al. 1998). The same argument may be applied to land application of biosolids, however, there is little data on the concentrations of *B. cepacia* in biosolids and it is unknown if land application programs significantly affect background populations that naturally occur in the environment.

Algal toxins, produced by *Cyanobacteria* and other algae, have been included in the USEPA Drinking Water Contaminant Candidate list (USEPA 1998). This is a list of contaminants known or anticipated to occur in public water supplies and that may require regulation. They have not been included in Table 1 as agents of potential concern in biosolids because the conditions encountered in most biological wastewater treatment processes would not be conducive to the growth of these organisms. They require sunlight for growth, and the dense bacterial biomass in aerobic treatment processes would severely limit light penetration. Anaerobic processes occur in enclosed vessels, completely eliminating light. In addition, cyanobacterial toxins are biodegradable (Rapala et al.1994; Bourne et al. 2001), so even if introduced into wastewater from another source, they should be removed by biological treatment processes. While it is not expected that biosolids would directly serve as a source of algal toxins, uncontrolled runoff from biosolids application sites, or any other agricultural setting where organic or chemical nutrients are present, could impact nutrient loading in surface waters and potentially contribute to the conditions causing algal blooms.

One common thread with most of the emerging bacterial pathogens discussed here is that they do not fit the classic enteric bacteria, fecal-oral pattern, and they predominantly affect people with specific predisposing factors. The organisms themselves are frequently widely distributed in the environment, often in reasonably high concentrations. There are generally both non-pathogenic and pathogenic strains that may be difficult to distinguish by conventional testing methods. These issues make it difficult to assess potential impacts associated with waste management practices. Given the widespread distribution of the organisms in nature, it would appear from a public health perspective that intervention and prevention of these diseases need to be focused on the primary routes of transmission, *i.e.* critical control points.Waste disposal practices, such as land application of biosolids, should not exacerbate the control procedures or processes necessary to prevent illness.

The last two items noted in the "new issue" column of Table 1, endotoxins and antibiotic resistance, are directly related to bacteria or bacterial pathogens, but do not represent specific diseases *per se*. Many emerging and reemerging bacterial pathogens synthesize toxins that serve as primary virulence factors. Another category of bacterial toxins, the endotoxins, are a group of nonspecific lipopolysaccharide-protein complexes derived from the outer layer of gram-negative bacterial cell walls (DiLuzio and Friedman 1973). These toxins are not related to a specific disease, but may produce a variety of physiological responses, including elevated body temperatures, coughing, breathlessness and flu-like symptoms. Endotoxins have been measured in air at composting plants and some studies have indicated that compost workers may experience increased upper airway inflammation related to bioaerosols (Clark et al. 1983; Douwes et al. 2000; Van Tongeren et al. 1997) . This has also been observed at facilites composting green waste containing no sewage sludges (Weber et al 1993). Endotoxins are also associated with decaying grain, hay and silage dusts on farms (NASD 1992). The concentration of contaminants, and health effects observed in some compost workers, are similar to those observed in workers in other agricultural settings (Weber et al 1993). Medi-

cal studies have been recommended to learn more about the effects of this type of occupational microbial exposure. Some form of respiratory protection appears appropriate for workers in areas with high levels of organic dust production. Dutch policies encourage home composting of organic waste such as banana skins, potato peels, etc. Researchers reported that in-home composting bins increased endotoxin levels in the home (ENN 2000). Some people with respiratory illnesses said their symptoms got worse, but there was no medical documentation of these anecdotal reports. Just as many of the emerging pathogens are commonly found in the environment, so too are endotoxins. Specific health effects are related to the concentration of endotoxin one is exposed to, and the sensitivity of the individual. There are no data relating off-site health effects from endotoxins associated with land application of biosolids, manures or other organic agricultural soil amendments, and potential health effects appear to be primarily related to high level occupational exposures. Low concentrations of endotoxins were detected in groundwater at two wastewater land disposal sites; column studies with sewage demonstrated that 90 to 99 percent of the endotoxin in sewage was removed by travel through 100 to 250 cm of soil, but suggested heavy rainfall might mobilize movement of endotoxins (Gerba et al. 1980). No data were found relating endotoxins in groundwater and land application of biosolids or other organic soil conditioners.

The increasing rate at which microorganisms are developing antibiotic resistance may represent one of the most serious emerging threats facing modern medicine. The world may soon be faced with previously treatable diseases which have again become untreatable, as in the pre-antibiotic era. Multiple antibiotic resistant organisms have become a serious threat in health care facilities and are increasingly appearing in new settings. The complex topic of antibiotic resistance is beyond the scope of this paper. Recognizing the potential of this threat, a consortium of ten federal agencies and departments, lead by the Centers for Disease Control and Prevention, joined forces to develop a "Draft Public Health Action Plan to Combat Antimicrobial Resistance" (CDC Online 2000). Addressing this problem will not be simple and will involve complex and controversial scientific, medical and economic issues. One recommendation in this comprehensive document is to "Conduct pilot studies to assess the extent and impact of environmental contamination by antimicrobial drug residues and drug-resistant organisms that enter the soil or water from humans and animal waste. If appreciable contamination is detected, conduct routine or sentinel surveillance in waste, in surface and ground water, and in soil from agricultural areas in which waste is used for fertilizer." (CDC Online 2000). What role current waste disposal practices, including land application, may play in the larger picture of spreading antibiotic resistance is unknown, and addressing the question will be difficult.

Panel participants at the 1983 Denver workshop reached consensus on many issues concerning land application of domestic waste with regard to pathogen risks, and also recommended areas of needed research. It appears appropriate to now reexamine these findings in context of the above discussion and in relation to current concerns. The following conclusions from the "pathogen panel" members encompass pathogens in general and were not limited to bacterial pathogens.

1983 Panel Conclusions

1. "With proper management and safety allowances based on research data, land application is a safe, beneficial and acceptable alternative for the treatment of municipal wastewater and sludge."

In the author's experience, most agencies, businesses and regulators involved with land application of biosolids would argue this remains an accurate assessment and that the 503 regulations were scientifically based and adequately defined what constitutes proper management and safety allowances. Many would also agree that there may be room for discussion about practice, i.e., how well the actual handling and practice of land application programs comply with the intent of the regulations. Addressing this to some extent, a manual of best practice was developed for storage of biosolids (USEPA 2000). This general view of the adequacy of the 503 regulations is not universally shared by the public or academic community, and vocal and organized public opposition to land application, particularly with some Class B products, is growing in many areas of the U.S. In some cases local jurisdictions have passed, or are contemplating, bans on the use of Class B materials, even when applied in full compliance with the 503 regulations.

2. "At the time of the workshop, in the US there are no known outbreaks of infectious disease attributable to land application of wastewater or sludges."

Although there have been anecdotal stories of people becoming sick, and even litigation alleging harm, there are still no documented disease outbreaks associated with, or traceable to, land application of treated biosolids.

3. "Major improvements in monitoring methods, especially in the virus area, have been achieved. Data generated during the decade since 1973 provided the scientific basis for criteria at the federal level."

A discussion of viruses is presented elsewhere in these proceedings, however, this conclusion remains valid. Virus data generated were considered adequate to develop treatment criteria, and subsequently the 503 regulations. Molecular technology now offers potentially more sensitive detection methods, however, these methods may detect non-infectious viruses or genetic fragments; experimental design and data interpretation are critical with the use of these techniques.

4. "In terms of current detection capability, federal sludge disposal criteria are adequate to protect public health from pathogenic microorganisms. Data currently being generated will provide guidance to any consideration for relaxation of the criteria. Criteria and management guidance based upon geographical considerations are warranted."

At the time of the 1983 workshop, the criteria and management guidance were based on process standards referred to as Processes to Significantly Reduce Pathogens and Processes to Further Reduce Pathogens. These processes had been documented through extensive research to produce certain levels of pathogen inactivation. A subsequent field survey (Yanko 1987), which will be discussed in the next section, found that these treatment processes were not being uniformly or consistently operated and that product quality was quite variable. As a result, the 503 regulations incorporated performance criteria in addition to process standards to document process performance. The 503 regulations do address some geographical issues associated with land application, such as snow and associated snow-melt, but in general geographical considerations (e.g., population density/land available for use or disposal of biosolids; depth to groundwater, etc.) were not considered in the regulations.

1983 Panel Recommendations

1. "Determine survival and regrowth of established human pathogens (e.g., *Salmonella)* and newly recognized waterborne pathogens (e.g., *Campylobacter, Yersinia*) in sludge and soils amended with sludge and wastewater."

Some research has been conducted to examine *Salmonella* regrowth in biosolids (Sidhu et al., 2001; Hussong et al. 1985). The greatest potential for regrowth may occur when biosolids are blended with other nutrient-containing substrates to formulate soil amendment products (Yanko 1987; Skanavis and Yanko 1994). Incorporating a holding period before distribution of blended, bagged products was shown to control the problem (Yanko unpublished data). There has been little or no research examining the potential for other enteric pathogens, such as *Campylobacter* or *Yersinia* to repopulate in treated biosolids. The ability of *Yersinia* to grow in cold conditions presents a novel opportunity for this organism to potentially regrow. This question will also be further discussed later in this paper.

2. "Improve methods for detection, enumeration, and assessment of virulence of these pathogens in sludge and wastewater."

There has not been extensive focused research in the U.S. conducted specifically aimed at improving biosolids testing. On the other hand, the revolution in biotechnology has opened up analytical options that, if properly used, should help address some of the questions about the fate of pathogens and supplement past research.

3. "Determine relevance of existing indicator organisms as indicators of the presence of new pathogens."

This recommendation remains as valid today as it was in 1983. Little has been published relating the reliability of traditional indicators to predict the presence of emerging pathogens.

4. "Identify factors and determine survival and translocation of pathogens and indicator organisms in sludge amended soils under different infiltration rates and in different soils."

Much research has been conducted examining movement of pathogens in soil, especially in context of groundwater contamination. Most of that effort, however, has not focused on biosolids land application sites. There does not appear to be good scientifically based guidance to address questions such as minimum depth to groundwater at a biosolids land application site.

Occurrence of Pathogens in Distribution and Marketing Municipal Sludges, 1987 Study for USEPA

This two year study was conducted in the writer's laboratory (Yanko 1987) and the resulting data were used extensively in the final formulation of the performance criteria for Class A pathogen reduction defined in the 503 regulations. The study examined the microbiological quality of end products that were being

distributed for home use or used in applications where the potential for public exposure was greater than that expected for purely agricultural uses with non-food crops. Samples were collected from 26 different cities around the U.S. Two facilities, one on the east coast and one on the west coast were sampled weekly for a year. The other 24 facilities were sampled every other month (6 samples at each site) during the year. The majority of the products tested were treated by processes subsequently defined as Class A processes. Nine of the 26 locations treated sludges by a combination of either aerobic or anaerobic digestion followed by air-drying.These processes are classified as Class B processes under current regulation. Products were tested for indicator bacteria, fungi, bacterial pathogens, parasites and viruses (Table 3).

Table 3. Microorganims Selected for Analysis in Occurrence of Pathogens in Distribution and Marketing Municipal Sludges (Yanko 1987)

Indicator Groups	Pathogenic Microorganisms
Total coliform	Enterotoxigenic *E. coli*
Fecal coliform	Total enteric bacteria
Fecal Streptococci	*Salmonella*
Aerobic plate count	*Campylobacter*
Anaerobic plate count	*Yersinia*
Total fungi	*Ascaris* **ova**
Thermophilic fungi	Total parasites[1.]
Bacteriophage	Enteric viruses[1.]

[1.] See original reference for definitions and analytical methods of detection

The 1987 Project Conclusions

1. All of the sewage sludge products examined were found to contain variable densities of indicator microorganisms. Some products contained bacterial pathogens at high frequencies and levels. Variability of microorganism concentrations was often great between different facilities and between different samples from the same facility. Many of the observed trends would not have been detected without a large number of samples collected over a long period of time.

This first statement presented an overview of the findings and reflects concentrations of microbial densities, as measured by nonspecific assays such as heterotrophic plate count populations, fermenting and nonfermenting bacterial populations, and fungal densities as well as specific bacterial pathogens. As noted later, viruses and parasites were not found to be a concern at any of the facilities or end products tested, based on the analytical methods used.

2. Overall, the highest concentrations of microorganisms occurred in samples from static pile composting systems; the lowest concentrations were found in pelletized sludge from a heat drying process. Microorganism densities in aged anaerobically digested-air dried sludges were as low as, or lower, than most of the composted sludges.

This statement again reflects both general measures of microbial density and specific bacterial pathogens. Seven static pile composting facilities were included in the sampling program. Some of the consistently highest quality products tested came from specific static pile facilities while, in contrast, some of the poorest quality products came from other static pile facilities. When the data were averaged, the static pile facilities faired poorest overall as a group, although specific sites were excellent. This is not an indictment of the static pile process. The results did indicate that the process was not being consistently operated and controlled at different facilities.

3. Composts modified with various materials to produce commercial soil amendments contained significantly higher concentrations of bacteria and fungi than the base compost material. The data suggested a nutrient-related regrowth phenomenon.

As noted, this study did not evaluate processes, *per se*, in that only end products were tested. It generally stood out, however, that composts that were modified after treatment by addition of other organic components to produce blended soil amendments, had significantly higher densities of the broader measures of microbial density and also had a greater probability of containing *Salmonella*.

4. Potentially pathogenic bacteria including *Salmonella sp.*, *Yersinia enterocolitica* and toxigenic *E. coli* were detected. *Salmonella sp.* were the most frequent pathogens detected. The quantitative test for toxigenic *E. coli* indicated that these strains, when present, occurred at very low levels. However, the percentage of colonies that was toxigenic strongly suggested that the concentration of toxigenic strains was much higher than indicated. *Yersinia enterocolitica* occurred at very high densities in some samples. The isolation of *Yersinia* was consistent with a seasonal occurrence. The prevalence and density was higher in colder months. Based on a small number of tests the *Yersinia appeared t*o be avirulent (not causing disease).

This finding is probably the most pertinent to our current discussion and concerns. Of the three enteric pathogenic bacteria mentioned, the analytical procedures for detecting *Salmonella* were the most reliable. The study showed that *Salmonella* was frequently detected in final products, often at reasonably high densities, and that there was a clear relationship between the concentrations of indicator bacteria and the probability of *Salmonella* being detected. This relationship was later used to develop microbial limits for Class A products.

At the time the study was conducted, methods for detecting toxigenic *E. coli* and pathogenic *Yersinia enterocolitica* were complex and time consuming. Toxigenic *E. coli* were detected by screening *E. coli* isolates for toxin production in a mammalian cell culture assay, the Y-1 adrenal cell assay. During the study, positive fecal coliform test tubes from multiple tube dilution tests were streaked to Endo agar for isolation of *E. coli*. Typical *E. coli* colonies were picked and tested for toxin production. Tubes from the original MPN test were scored positive when a toxin positive isolate was detected, and then an MPN value was computed. This approach to quantifying the toxigenic *E. coli* indicated that densities were very low. The problem with the approach was that only the organisms present in highest numbers in a positive MPN tube were likely be detected when the culture was streaked and a small number of colonies picked. For example, after incubation a positive culture tube might contain 10^5 "regular" *E. coli* per mL and 10^3 toxigenic *E. coli* per mL. When that culture was streaked for isolation, simple distribution dictated that it would be unlikely to detect one of the toxigenic strains.

Recognizing this problem, the project data was reexamined using another approach. A little more than 7,000 individual *E. coli* isolates were randomly picked and tested for toxin production during the study and 0.32 percent of these were found to produce toxin. If one assumes that the toxin producing strains grew at the same rate as other *E. coli* strains during culture in the Lauryl tryptose broth and E.C. medium used for the fecal coliform test, then picking of isolated colonies represented random sampling and the percentage of toxin producing *E. coli* detected represented their distribution in the original sample population. If we accept that 0.32 percent of the *E coli* population in sewage-derived material may be toxigenic, that translates to the potential for a Class A product to theoretically contain up to 3 toxin producing *E. coli* per gram. That is slightly higher than the limit for *Salmonella*, but probably represents a relatively insignificant risk. In contrast, Class B anaerobically digested biosolids, receiving no further treatment, could theoretically contain 6,400 toxin-producing *E. coli* per gram. It needs to be emphasized that this is a theoretical exercise based on specific assumptions. The fact remains that toxin-producing strains of *E. coli* were detected. Additional research is warranted to verify the true numbers of toxin producers and fate of these organisms in biosolids and land application. The impact of the highly virulent *E. coli* 0157:H7 strain in recent years adds to the importance of this question. In perspective, the same question holds true for animal manures which for the most part are not regulated.

Yersinia enterocolitica is similar to *E. coli* in the sense that most environmental strains are nonpathogenic. The standard tests used to measure *Y. enterocolitica* do not distinguish between the pathogenic and nonpathogenic strains. During this study very high concentrations of *Y. enterocolitica*, up to 10^7 per gram, were detected in some compost products that were derived from Class A processes in locales with cold winters. The concentrations of *Y. enterocolitica* showed a seasonal trend with highest concentrations occurring during cold weather, as shown in Figure 1. This observation is consistent with the ability of *Y. enterocolitica* to grow at low temperatures. It should also be noted that CDC data indicates most cases of foodborne *Y. enterocolitica* occur during winter (MMWR 1998), again suggesting a climatic link.

Methods available in the 1980s to distinguish pathogenic *Y. enterocolitica* were complex, time consuming and expensive. The Yanko (1987) study contracted with the New York State Health Department to test selected isolates of *Yersinia enterocolitica* detected in biosolids for pathogenicity. Twenty-eight randomly selected cultures were thoroughly characterized using 36 biochemical marker tests and they were serogrouped with antisera 0:1 through 0:34. None of the isolates were pathogenic based on the virulence markers. While these results were encouraging and suggested the *Yersinia* present were environmental strains, that observation must be tempered by the fact that only 28 isolates were characterized from materials that

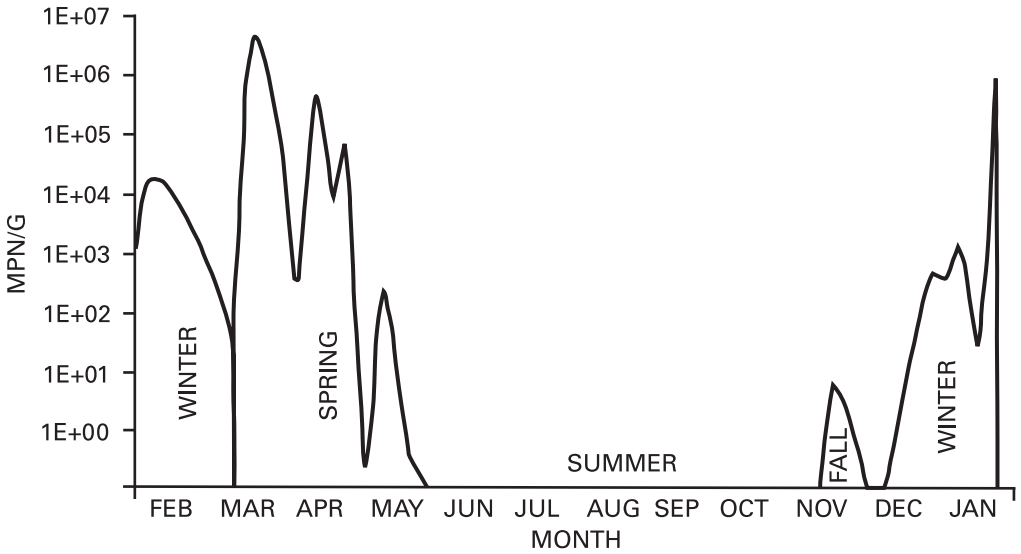

Figure 1. Seasonal occurrence of Y. enterocolitica at a compost site in northeastern U.S. (Yanko 1987)

contained as high as 10^7 organisms per gram. Newer molecular-based test techniques have provided tools to revisit this question and more definitively determine the potential for biosolids to serve as a reservoir of pathogenic *Y. enterocolitica*.

5. No significant health hazard was found associated with respect to *Campylobacter*, parasitic helminth ova or enteric viruses. The test for campylobacters in compost was relatively ineffective but other available data suggest these bacteria could not survive composting or air drying. Helminth ova were detected regularly but no indications of viability were observed. No protozoan cysts were found.

Seeding experiments and "blind spike" experiments conducted as part of the quality assurance for the project indicated that *Campylobacter* was not recovered well, and the absence of *Campylobacter* in samples needed to be interpreted with caution. As mentioned earlier in this paper, available literature and "conventional" wisdom suggested the fastidious nature of this organism made it unlikely that it would be hardier than other pathogenic enteric bacteria in biosolids. The high incidence of *Campylobacter* food poisoning now recognized suggests this assumption should be reexamined. There is still no evidence to suggest *Campylobacter* would survive Class A inactivation processes. The fate of *Campylobacter* during anaerobic digestion and subsequent land application appears to warrant additional research.

In contrast to *Campylobacter*, the project quality assurance (QA) for *Ascaris* ova was quite good. Recoveries of spiked ova averaged 88 percent in the type of samples being analyzed and "blind spikes" were consistently detected during the study, suggesting the data and conclusions about helminth ova were robust. In contrast, the QA for protozoan cysts, specifically *Giardia*, was much like that for *Campylobacter*, i.e., recovery of spiked cysts was <1 % and "blind spikes" were often not detected. Nevertheless, it was concluded that cysts were inactivated based on the helminth ova data. Available data continues to suggest that helminth ova are a good surrogate for cyst inactivation. There are also no data indicating that human viruses are resistant to Class A inactivation processes (see Chapter VI).

6. The fungus *Aspergillus fumigatus* was detected in products from most sample sites, but usually at low densities. The highest concentrations of *A. fumigatus* occurred in composts from static pile composting facilities.

Concerns about *A. fumigatus,* and bioaerosols, were thoroughly reviewed by Millner et al. (1994). During the Yanko (1987) study, *A. fumigatus* was generally detected in all biosolids products tested. This was expected given the ubiquitous nature of this fungus. The densities of *A. fumigatus* were greatest, by far, in samples from static pile composting facilities. It was hypothesized that this was related to the common practice of screening out the wood chips used as a bulking agent and reusing them with fresh sludge. This essentially seeds the sludge with large numbers of *A. fumigatus* at the start of the composting process. The other composting processes sampled during the project did not recycle bulking agents and typically had much lower concentrations of *A. fumigatus* in the final product. This speaks to a larger question from a

management perspective. Different treatment processes have advantages and disadvantages. One process may be more prone to odor problems, but very efficient at high rate pathogen inactivation. Another, as suggested for static pile, may have a greater tendency to produce higher concentrations of *A. fumigatus.* It is important to understand the pros and cons of each process in order to select the appropriate system for a given circumstance, and then effectively manage that process to prevent negative impacts.

7. Given the considerable variation observed in microbial densities and the reasonably frequent isolation of *salmonellae,* bacterial monitoring to assure product quality may be of value for the home use of sludge and compost soil amendments. Regression analysis suggested that total or fecal coliforms or fecal *streptococci* may be suitable indicators for monitoring.

This study was conducted before the 503 regulations were adopted and there were no final-product microbial performance criteria recommended at the time. This conclusion resulted in a specific recommendation, as noted in #7 below, suggesting that bacterial limits be established.

8. The occurrence of pathogenic bacteria in distributed and marketed municipal sewage sludge products represents a potential health hazard. However, the extent of risk associated with use of such products remains to be determined.

This conclusion was based on the concentrations of specific pathogens, especially *Salmonella*, detected in many tested products. The subsequent 503 regulations for Class A products were formulated to essentially eliminate the potential risks revealed during this study.

1987 Project Recommendations

1. Factors associated with the extensive variability observed in the microbial populations need to be better delineated in order to institute appropriate control measures.

While it is possible, and most likely probable, that some process reliability studies have been conducted by some agencies, that information is not readily available in the literature.

2. Significance of the relatively high microbial concentrations in static pile compost products should be determined. The influence of recycling wood chips should be further evaluated.

The adoption of performance limits, *i.e*. specific bacterial limits for Class A products, was intended to address the issue of reliability.

3. Additional studies on *Salmonella* regrowth are recommended. The effects of substrate additions should be evaluated. Laboratory regrowth experiments to date may not have adequately simulated field conditions.

There have been studies conducted on *Salmonella* regrowth. There does not appear to be a consensus on the extent or significance of this question.

4. Consideration should be given to establishing criteria and conducting research necessary for qualifying digested, air dried sludges as equivalent to PFRP treated sludges.

Considerable effort has been devoted to this question in specific venues, but there has not been resolution of the question. It nevertheless was quite apparent from the Yanko (1987) data that, from a microbiological perspective, it is inappropriate to group air-dried solids that have been stockpiled and stored for long periods of time, (1–2 years minimum), in the same category as anaerobic digester biosolids.

5. Further studies are recommended to quantitate toxigenic *E. coli* populations. Gene probe techniques may be applicable to this task.

Molecular techniques have developed and improved since this study was conducted, but this question has not been addressed.

6. The potential for sludge and compost to serve as a reservoir of pathogenic *Yersinia* in certain locations needs additional evaluation.

The study data suggested potential concerns about *Y. enterocolitica* are greatest in geographic areas that have cold winters. The potential for pathogenic *Y. enterocolitica* to populate biosolids during cold weather is still unknown.

7. Bacterial limits may need to be established for the uncontrolled home use of sludge and compost products or appropriate educational material should be supplied to users of the products.

The 503 regulations established performance criteria and set bacterial limits as suggested.

8. Studies should be conducted to determine the extent of risk, if any, of bacterial infections from the use of distributed and marketed municipal sewage sludge products.

As already noted, the 503 regulations were formulated to eliminate the potential risks suggested by this study. There have been no health studies conducted to examine theoretical risks to users of distributed or marketed biosolids products for home use.

Impact of Methods on Value and Utility of Data

The safety of land application programs and the underlying data used to develop the 503 regulations have been challenged by some on the basis that the analytical methods used were inadequate to make sound judgements. There is little doubt that analytical methods are in a constant state of flux and new methods with greater sensitivity continue to be developed. This trend has been acutely apparent with the analysis of chemical constituents as we progressed from parts per million to parts per billion and now to parts per trillion detection levels. Most of the microbiological data available for the occurrence and fate of microorganisms in biosolids are based on culture-based methods for bacteria, fungi and viruses, or direct microscopic exam for parasites. The reliability and sensitivity of the methods that have been used have undoubtedly been quite variable over the many years and myriad of studies that provided the basis for the 503 regulations. However, a sweeping indictment of methodology is clearly inappropriate. That is readily apparent by examining a portion of the extensive quality assurance data (Tables 4 and 5) that was reported with the Yanko (1987) study.

Table 4. Percent Recovery of Spiked Organisms for Methods Determined before Start of Yanko 1987 Study

Test	Percent Recovery
Salmonella	105
Campylobacter	<1
Yersinia enterocolitica	109
Ascaris **ova**	88
Giardia **cysts**	<1
Enteric viruses	30

Table 5. Detection of Blind Spikes During Conduct of Yanko 1987 Study

	Number of blind spiked samples:	
Organism spiked	Submitted	Detected
Salmonella spp.	2	2
Salmonella typhi	1	1
Yersinia enterocolitica	6	6
Campylobacter jejuni	5	3
Shigella soneii	1	0
Toxigenic *E. coli*	4	2
Ascaris ova	6	6
Giardia cysts	8	3
ECHO II virus	2	1
Polio 1 virus	2	2
Coxsackie B4 virus	2	2

Some methods performed quite well, while others did poorly. While individual tests may have not performed well, the body of data was adequately robust to permit sound judgements. For example, as mentioned earlier in this paper, the test for *Giardia* cysts did not perform well. On the other hand, the test used to detect *Ascaris* ova did provide reliable data for the types of samples examined, based on the recovery experiments and blind spike detection rate. There are sound reasons to believe that *Ascaris* ova are more resistant to inactivation, so the consistent demonstration that *Ascaris* ova were dead renders the poor performance of the *Giardia* test somewhat moot. It is highly unlikely that *Giardia* cysts could survive a process that inactivated *Ascaris* ova. Similar analogies can be made in other areas. Experimental design can also compensate, to some extent, for low recovery or sensitivity. If sensitivity is low, but precision is acceptable, inactivation rates and removal efficiencies can be reliably determined. Each study needs to be examined on its own merits to understand and interpret the degree to which analytical methods may have been a factor in the results of the study. Unfortunately, quality assurance data are seldom available to help make that assessment.

Compliance monitoring represents a different set of concerns. For compliance testing it is critical that the methods used have been validated for all types of samples that will be tested and that appropriate quality assurance and performance criteria have been established to document laboratory performance. While most laboratories performing coliform testing on water samples will have appropriate QA programs in place for that analysis, specific analytical performance criteria have not been established for biosolids testing. Performance testing programs are not available for biosolids testing, as with water analyses, and certification programs specifically for biosolids testing have not been established. The 503 regulations also permit compliance with Class A criteria by testing for *Salmonella* instead of fecal coliform bacteria when approved processes are employed, or by testing for fecal coliform bacteria/*Salmonella*, *Ascaris* ova, and enteric viruses in the absence of an approved process. Multiple laboratory validation testing has not been conducted for *Salmonella* testing or *Ascaris* ova testing, and there is evidence suggesting the 503-specified compliance methods may not be adequately sensitive to meet the spirit and intent of the regulations (Yanko et al. 1995). These issues are most pertinent to the Class A criteria. Although standardized biosolids-specific quality assurance programs are lacking, the experience most laboratories have with coliform testing and the basic QA programs in place for that test suggest that Class A compliance by fecal coliform most likely achieves the basic goal of verifying treatment process performance. In the absence of laboratory performance data for pathogen analyses, the reliability of Class A compliance by pathogen analyses is uncertain.

Summary

Many of the pathogens of emerging or reemerging significance are bacteria. While it remains true that there are no documented cases of negative health impacts related to any of these organisms with the land application of biosolids, it is also true that there are few data documenting the fate of many of these organisms. Other factors also enter into an assessment of concerns about the bacterial pathogens, such as the potential for them to grow or repopulate and questions about developing antibiotic resistance. Clearly additional research will be needed to answer these questions and verify the adequacy of current regulations and policies, especially in relation to the use of Class B biosolids. Even within existing regulation, the Class B designation is misleading from a microbiological perspective. Some Class B products are of equivalent microbiological quality to Class A products, but expensive, complex microbiological analyses are required to document that quality in the absence of an approved treatment process. These kinds of discrepancies can only serve to add to public confusion about the meaning of the regulations and the Class A vs. Class B distinction. Although additional research is warranted to examine specific questions, including the compliance testing methods themselves, there are no data at this time to suggest that land application and utilization of biosolids as a recyclable resource cannot be practiced in a safe and environmentally sound manner, or that current practices have caused adverse health effects. Public perception and public acceptance may prove to be a much greater hurdle.

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Bacteria–Sludge/Biosolids Workgroup: Discussion Summary, Conclusions and Recommendations

Introduction

The focus of this workgroup was emerging bacterial pathogens in sludges and biosolids. This workgroup also considered new issues of concern for general infectious disease agents that may pose threats to public health. The workgroup considered the amount and quality of data relating to a number of bacterial species, as well as their potential threat to public health, in order to identify deficiencies in our knowledge and to prioritize research needs.

Participants in this workgroup were: William Yanko, Leader; Mark Meckes, Facilitator; Bob Brobst; Jeffrey Burnham; Rebecca Bushon; Timothy Ford; Paul Gale; Terry Logan; Don Reasoner; Mansour Samapadour; and Catherine Simmonds.

Organisms

General Issues

The workgroup discussed general issues involving pathogens and bacteria in sludge and biosolids that have reached our awareness since a number of federal agencies and academic institutions last made a comprehensive assessment of the issues and research needs associated with pathogens in sludges and biosolids in 1983 (Page et al. 1983). These issues included the social, biological, technological and public policy-oriented factors that may have an impact on how infectious disease research and disease control are addressed in the near future. One of the major social factors affecting transmission of disease agents is the ever-increasing quantity of travel within the U.S. and worldwide. An area of concern with increased travel is the potential difficulty in surveillance of disease across geographic boundaries, particularly for those pathogenic agents that do not result in especially visible or immediate symptoms of disease in human carriers or other host organisms. Theoretically, these pathogens could effectively disappear within communities and experience extended periods where transmission between individuals goes undetected. Other social and technological factors that influence transmission of disease and that were discussed by the group included increased mass food production, increased recognition of immunocompromised populations and, of particular concern, the widespread use of antibiotics.

The indiscriminate use of antibiotics has received more attention in recent years due to their potential for changing the genetic makeup of the bacterial pathogens. Although some degree of control has been effected over certain disease agents by taking advantage of natural environmental limitations of the organisms with regard to temperature, pH, or oxygen regime, antibiotics have become a popular tool for reducing mortality due to infectious diseases and improving quality of life in industrialized nations. The use of antibiotics has become relatively commonplace in the U.S., primarily through medical and veterinary applications, but also increasingly by their incorporation into personal care products and household cleaners. Recently, threats to public health from 'overuse' of antibiotics have been communicated to the public, the message being that there is evidence of bacterial pathogens developing resistance to traditional antibiotics, perhaps at faster rates than the scientific community had realized (CDC 2001 and FDA 2001). The workgroup discussed the process of selection of antibiotic resistance in pathogen populations exposed to the antibiotics including acquisition of genes for resistance, decreased survival of those individuals that do not have genes for resistance, and increasing prevalence of individuals with antibiotic resistance over successive generations. Information was available to the workgroup suggesting that high levels of transferred resistance factors occur among bacteria and that antibiotic resistant microorganisms are relatively stable.

Bacterial Pathogens of Concern

The workgroup reviewed the list of bacterial pathogens and issues of potential concern presented by W. Yanko (these proceedings, Yanko Table 1). From this list, twenty bacteria that were considered to be of

cern in 2001 were selected for a focused review of data availability for each organism. The list developed by the workgroup (Table 1, below) included those bacteria thought to be of important in terms of their public health significance (based on the number of people that may be infected and/or the severity of the disease), their prevalence in sludges and biosolids, and their survival during treatment processes. The survival during wastewater treatment was considered to be particularly important. The group recognized that there were many bacteria that have the potential to enter the sewage system and that by focusing on those best able to survive treatment processes, the list should include those that will be of most concern in biosolids. The paper by Yanko (these proceedings) gives a detailed description of the issues associated with many species on the list. Using a consensus process, the workgroup participants rated each of the 20 bacteria according to the following parameters: infectious dose; occurrence in sludges; survival through treatment and in the environment; analytical method; data rating; public health significance and heartiness. The rating system is explained in more detail in the footnotes accompanying Table 1.

The workgroup evaluated the organisms overall, but did not rank them. Ranking the organisms by summing the last three columns would be inappropriate since each of these factors have unique implications. For example, a high number in the "data rating" column means only that we have considerable data regarding that organism. A high number in that column with a low public health significance number and a high heartiness number might yield a good indicator organism, but would not necessarily be considered more important than another organism with a higher rating for public health significance.

Changes between the 1983 list (Page et al. 1983) and the 2001 list of bacteria of concern in biosolids included: 1) *Vibrio cholerae* was no longer considered to be of major concern because of the small number of cases that occur in the USA. *Leptospira* was also considered to be of minor significance for the same reason. 2) A wider variety of *E. coli* are now considered to be significant, reflecting the increasing knowledge about this species. 3) *Helicobacter pylori, Neisseria meningitidis, Burkholderia* spp, *Mycobacterium* spp. and *Aeromonas* spp. were added to the list, reflecting increased understanding about the role of these species in diseases.

Some species remained on the 2001 list, although after almost twenty years they could not technically be considered emerging pathogens. Staphylococci, streptococci and clostridia for example, are known pathogens that were included in the list because they were considered to be potentially useful indicators of pathogen die-off. The group also recognized that many of the species included on the list were not emerging pathogens as such but that social and technological changes meant that there were new reservoirs for already known pathogens.

Following their scoring effort, the workgroup was able to organize the list of bacteria from increasing to decreasing data availability and quality by collectively using the scores placed in each of the columns. For those emerging pathogens with a public health significance rating of 5 or higher (arbitrarily selected by the workgroup as being of greatest interest or importance), it appears that *Salmonella* and *E. coli* are the best-documented species, while *Pseudomonas* spp. and *Helicobacter pylori* are the most data-poor of the group. The workgroup did not consider *Aeromonas* or *Neisseria meningitides* to be significant public health threats when compared to *Psuedomonas* spp. and *Helicobacter pylori*.

Other Organisms

The workgroup also considered organisms that had not been covered by the keynote presentations, namely fungi. The workgroup discussed the risks posed to workers exposed to fungi in composting heaps. Workgroup members were concerned that there may be data gaps regarding airborne bacteria/fungi generated during turning of compost heaps. They thought that while the quantity of *Aspergillus* is known, controls are needed. Yeast was considered to be common in biosolids, but is possibly only a public health concern for immunocompromised populations.

Bacterial Organisms Conclusions/Recommendations

The workgroup considered a number of social and technological factors that influence transmission of disease and concluded that the following are of importance: Increased travel and associated difficulties of disease surveillance across geographic boundaries; Increased mass food production; Increase in immunocompromised populations; and Widespread use of antibiotics.

Twenty bacterial organisms were considered to be of concern in municipal sludges and biosolids. These organisms were rated by consensus according to several parameters including infectious dose, data availability, survival during treatment and in the environment, and public health significance.

Table 1. Rating of Bacterial Pathogens of Concern by Availability and Quality of Data

Pathogen of Concern	Data Availability/Quality Parameters							
			Survival[c]					
	ID_{50}[a]	Occurrence[b]	Treatment	Environment	Analytical Method[d]	Data Rating[e]	Public Health Significance[f]	Heartiness[g]
Salmonella	+++	+++	+++	+++	+++	15	10	1
E. coli	N/A	+++	+++	+++	+++	15	0	1
E. coli strain O157	+	(+)	+	+	+	5	10	1
ETEC, EPEC, EIEC	?	?	-	?	+	0	7	1
Shigella spp.	+++	+	-	-	+	3	10	1
Campylobacter spp.	+	++	+	-	+	4	10	1
Yersinia spp.	+	+	?	?	+	1	7	1
Listeria spp.	(+)	+	+	+	+	5	6	1
Vibrio cholerae	+	rare	-	+	+	2	4	2
Leptospira spp.	-	rare	-	-	+	-2	3	2
Staphylococcus spp.	+	-	-	-	+	-1	3	2
Streptococcus (enterococci)	-	++	++	++	+	6	4	2
Clostridium perfringens	-	++	++	++	++	7	2	3
Mycobacterium (nontuberculosis)	-	-	-	+	+	-1	2	3
Aeromonas spp.	-	-	-	-	+	-3	1	1
Legionella spp.	+	+	-	+	+	3	7	2
Burkholderia spp.	-	+	-	+	+	1	3	2
Neisseria meningitidis	-	-	-	-	+	-3	4	1

continued

Table 1. Continued

Pathogen of Concern	Data Availability/Quality Parameters							
	ID_{50}[a]	Occurrence[b]	Survival[c]		Analytical Method[d]	Data Rating[e]	Public Health Significance[f]	Heartiness[g]
			Treatment	Environment				
Pseudomonas spp	-	+	+	+	+	3	7	2
Helicobacter pylori	-	-	-	+	+?	-2	5	1

[a]ID_{50}—Infectious $dose_{50}$ is the quantity of a specific pathogenic organism required to produce an infection in fifty percent of the exposed population. Those organisms with a considerable body of reliable dose-response data, such as salmonellae, were given a score of +++. Those organisms for which relatively less information was available were given + or ++ and those organisms for which little was known were given a minus (-). Range: - to +++

[b]Occurrence—The knowledge about the presence of bacterial pathogens in sludges and biosolids was similarly rated. Those organisms for which there is a significant body of reliable literature were given +++. Those for which there is little or no information were given a minus (–). Range: - to +++

[c]Survival—The extent of information about the survival of bacterial pathogens through wastewater treatment processes and in the environment following use of biosolids was evaluated. The information was ranked in the same way as ID_{50} and occurrence. Range: - to +++

[d]Analytical Method—The workgroup considered whether or not sensitive and reliable methods of analyzing wastewater and biosolids samples for each of the listed pathogens existed. In ranking the availability of methods the group considered both sampling methods and assay methods. Availability of method data and reliability (use of a given method by multiple laboratories) is indicated by plus signs. Range: + to +++

[e]Data Rating—The actual values are relative and not absolute. In order to summarize the availability and quality of information needed to assess the risk posed by each of the organisms on the list, the workgroup summed the pluses (+) and minuses (-) in the preceding five columns. (Question marks and comments such as 'rare' count as zero.) The rating number can be negative or positive, with increasingly higher positive numbers indicating greater data availability. Range: –5 to 15

[f]Public Health Significance—Each disease agent was scored from 0 to 10, with 0 indicating that the agent has virtually no public health significance and 10 indicating a high potential for threats to public health. This rating is independent of the pluses and minus in the first five columns. In defining the public health significance the workgroup considered both the severity of the disease caused and the prevalence of the disease. For example, Salmonella received a high score because it has the potential to affect a large number of people, while Legionella received a relatively high score because the disease it causes can be severe. Range: 0 to 10

[g]Heartiness—This factor relates to the relative ability of the organism to survive environmental stress and/or treatment processes. A rating of 3 indicates that there is sufficient data to suggest that the organism is capable of surviving when exposed to various stressors, while a rating of 1 would indicate that the organism would not be expected to survive when exposed to stressors. Range: 1 to 3.

Other organisms that do not appear on the list of 20 were also considered. Yeasts are common in biosolids and may be a public health concern for immunocompromised populations.

Indicator Organisms

The workgroup discussed indicators for the presence of pathogens and for demonstrating treatment effectiveness. Current indicator species, particularly fecal coliforms, can be used to generally monitor the treatment processes that result in Class A biosolids. However, the suitability of an indicator must be tested for different types of treatment. It cannot be assumed that one organism or group of organisms will be an appropriate indicator for all types of treatment.

- While indicators can be useful for monitoring Class A treatment processes, their use for indicating pathogen reduction following Class B treatment processes is of no value. Many sludges can be classified as Class B biosolids on the basis of their fecal coliform concentration without having undergone treatment.

- As noted above, fecal coliform densities can be useful for monitoring a treatment process, however, they cannot always be used to indicate the removal of pathogens. Fecal coliforms are plentiful in municipal sludges; they are easy to detect and quantify. A given pathogen may only be present in relatively low numbers, and they can be quite difficult to isolate and/or enumerate. In many cases it is assumed that pathogens respond to treatment in the same way as fecal coliforms. Even when indicator species have been much reduced by a treatment, there is no guarantee that a given pathogen of interest has been reduced to levels below detection.

The workgroup discussed Class A versus Class B sludge treatment processes. (See Table 2.) Class A processes yield biosolids that have a fecal coliform density of <1000/g (dry weight) or a salmonellae density of <3/4g (dry weight) and no detectable enteric viruses (<1 pfu/4g) or viable helminth ova (<1 ova/4g). Class B biosolids may have a fecal coliform density of $<2 \times 10^6$/g (dry weight), or have been treated via a specified treatment process such as anaerobic digestion, aerobic digestion, lime stabilization, or windrow composting. It has been shown that when properly operated, the Class B processes mentioned yield at least a one hundred-fold reduction in the number of fecal coliform bacteria and a ten-fold reduction in the number of enteric viruses (USEPA 1999). It was noted that sludges with low initial fecal coliform densities could achieve the numeric Class B standard without treatment. It was also noted that the high fecal coliform densities associated with Class B biosolids suggest that pathogenic strains are most likely present. Therefore, it should be assumed that Class B processes contain pathogenic microorganisms and that appropriate land application and management strategies must be utilized in order to minimize exposure while natural attenuation further reduces the number of pathogens. (Note: Additional comments on indicator organisms appear below in the sections on *Ability to Assess Risks and Detection/Analytical Capabilities*.)

The workgroup also discussed public health concerns related to the storage of biosolids. One concern is the ability to control access to a field where Class B sludge deliberately or inadvertently has been kept in the field, sometimes for 90 days or more. The regrowth potential of *Salmonella* spp. increases as the sludge ages, as demonstrated by one Australian study that documented regrowth of *Salmonella* when dewatered biosolids and composted materials were stored for a year. Rewetting biosolids resulted in a *Salmonella* bloom followed by die-off (Gibbs et al. 1995). One of the reasons for the regrowth appears to be reduction in the numbers of microorganism species that compete with *Salmonella*. Actinomycetes were one such competitor that showed reduction (Sidhu et al. 2001).

The workgroup concluded that there is a need for indicators of biosolids quality that are oriented more toward public health than toward effectiveness of the treatment process.

Table 2. Existence of Indicators for Detection and Treatment of Bacterial Pathogens in Sludges

Biosolids/Sludge Classification	Treatment Efficiency	Presence of Pathogens
Class A	Yes (defined process)—fecal coliforms	Yes (defined process)—fecal coliforms
Class B	No	No

Indicator Organism Conclusions/Recommendations

The workgroup reached the following conclusions regarding indicator organisms: 1) There are indicators for treatment efficiency and the presence of pathogens in Class A biosolids produced by defined processes. 2) Indicators are of no value for indicating pathogen reduction in Class B products. 3) There is a need for indicators of biosolids quality that are more oriented toward public health rather than treatment effectiveness.

Ability To Assess Risks

Setting maximum contaminant levels of bacterial pathogens must take into consideration risk to human health and the environment. Better risk assessments are needed in order to help define maximum contaminant levels that may be imposed on the regulated community. The workgroup suggested that more research should be done to examine the differences between transport of pathogens in different media (e.g. air, water, soil), and differences in propagation or survival of pathogens under various conditions/environments. Risk assessment is dependent on the availability of data that is of known quality and is complete with the information necessary for conducting risk analysis. Based upon the available information (see Table 1), it was the opinion of the workgroup that the only organisms for which sufficient data exists to conduct risk assessments are *Salmonella* spp. and *E. coli*. For salmonellae, infectious dose data (obtained retrospectively from outbreak studies) and data on survival following treatment and environmental stress were available. For *E. coli*, the challenge is to separate strains by virulence and outcome of disease (those that produce a severe response in an individual versus those which cause relatively mild cases of diarrhea). Small data sets are available for *E. coli* strain O157:H7, while larger data sets are available for other strains.

There is a long list of pathogens for which risks currently cannot be determined. The range of infectious dose for bacterial pathogens is very large, which suggests that each of the pathogens would need to be addressed individually. Furthermore, variations in climatic conditions will affect growth and survival of bacterial species. This implies that the time required for attenuation of specific strains could be quite variable following land application. An alternate approach to risk management would be to determine which pathogen(s) is consistently present in high densities in municipal sludge and is the most resistant to treatment and environmental stressors. Monitoring survival of this organism(s) following treatment would provide an indicator system that could be used to ensure that microbiological risks are minimized. Although this option is attractive in concept, there are a considerable number of difficulties in selection of such an indicator(s). We do not live in a pathogen-free environment — the occurrence of pathogenic microorganisms in nature is normal. For example, bacterial species which are the most resistant to sludge treatment processes and environmental stressors are those which are capable of forming endospores. However, endospore-forming bacteria such as *Clostridium perfringens* are quite common, and are easily found in soil samples. Since a background population exists, what density above background would result in a significant risk for individuals? Are other infectious pathogens present in biosolids when the *Clostridium perfringens* densities are above background soil densities? Are there sludge treatment processes that are more effective in destroying *Clostridium perfringens* spores than other pathogens? Obviously there are numerous questions surrounding the selection of an appropriate indicator organism(s).

Ability To Assess Risks Conclusions/Recommendations

The only organisms for which sufficient data exists to conduct risk assessments are *Salmonella* and *E. coli* spp.

Detection/Analytical Capabilities

Methods are currently available for detection and enumeration of bacterial pathogens in biosolids samples. Such methods typically make use of general and/or selective enrichment combined with selective culturing or polymerase chain reaction (PCR) and molecular identification techniques. These techniques are quite sensitive and are potentially very useful in testing sludge/biosolids matrices. However, to develop data on all potential pathogens of interest using such methods would be an expensive and daunting task. One alternative is to analyze for a limited number of pathogens. These would be selected based upon their resistance to treatment and environmental stressors, and their potential impact to public health. One concern for such an approach is that the public health significance of a species may change over time thus compromising such a strategy.

Salmonella spp. are consistently found in untreated municipal sludges. Many species of salmonellae are known pathogens, and enumeration of *Salmonella* spp. is included in current U.S. regulations (USEPA 1993) as an alternate approach to measuring fecal colifoms in Class A biosolids. It was noted that a recent study

showed that the modified semi-solid Rappaport Vasilliadis (MSRV) technique for enumeration of salmonellae in biosolids recovered a greater number of these organisms than did the methods specified in current federal regulations (Yanko, et al., 2001). It was emphasized that the precision of the MSRV method needs to be evaluated by inter-laboratory performance testing on reference samples.

The workgroup discussed the appropriateness of using salmonellae as an indicator of treatment process effectiveness. The fact that salmonellae are ubiquitous in biosolids suggests that observation of their fate following treatment is a good indication of pathogen reduction. However, the fact that they are more likely than some pathogens to be susceptible to certain treatments suggests that the absence of salmonellae in biosolids is not a clear indication that other pathogens are not present. Also, the workgroup suggested the possibility of using a method(s) that allows for a lower detection limit and/or a presence/absence monitoring approach. The utility of such an approach has yet to be demonstrated, and would require additional research. They also noted that the U.S. regulations (USEPA 1993) would need to be modified to direct laboratories to use any new (or revised) method for an indicator organism or pathogen analysis used for compliance monitoring.

A similar situation exists for use of fecal coliforms as an indicator of treatment effectiveness. This group of organisms is typically found in high densities in municipal sludges. However, the fact that they are more likely than some pathogens to be susceptible to certain treatments suggests that low densities or even the absence of fecal coliforms in biosolids is not a clear indication that pathogens are not present, or that they have been reduced to levels that would not be considered a risk to public health. Methods exist for enumeration of fecal coliforms in biosolids (APHA 1998). However, these methods have not been evaluated by inter-laboratory performance testing on reference samples. The EPA plans to conduct such an evaluation.

Sampling

According to the U.S. 503 sludge regulations (USEPA 1993), the minimum number of biosolids samples to be collected for the purpose of determining microbiological quality is based upon the annual mass produced at a facility. The requirement of one sampling event per month for production of over 15,000 tons of material per year was thought to be insufficient. It was suggested that the sampling frequency should not have to be increased if frequent process monitoring could be shown to adequately ensure that the desired reduction in pathogens was consistently being achieved. One of the concerns with the sampling regime is whether or not biosolids can be released before the results of the testing are in hand. Release of the product should be based on the results of the assay.

Some of the sampling needs identified included the following:

Process control and monitoring: Testing of the product should not be considered a proof of the process. While indicators can be useful for monitoring Class A treatment processes, their use for indicating pathogen reduction following Class B treatment processes is of no value. Many sludges can be classified as Class B biosolids on the basis of their fecal coliform concentration without having undergone treatment.

Pathogen testing: There are a limited number of municipal and commercial laboratories conducting microbiological analyses of biosolids.

Frequency: Sampling frequency should be increased or correlated to frequently monitored process parameters (see above).

Quality Assurance: Develop a laboratory certification program so that municipal and commercial laboratories employ standard assay procedures and appropriate quality assurance measures.

Detection/Analytical Method Conclusions Recommendations

Workgroup conclusions/recommendations regarding detection/analytical methods are as follows: 1) Although there are methods for enumerating fecal coliforms in biosolids, these methods have not been evaluated by inter-laboratory performance testing on reference samples. 2) The precision of salmonellae enumeration methods also need to be evaluated by inter-laboratory testing on reference samples.

Processing/Control Technologies

The efficacy of Class A biosolids treatment processes was considered by the workgroup. Thermal processes designed to ensure that solids are exposed to elevated temperatures for adequate time intervals were considered to be adequate for destruction of pathogenic bacteria. Variations of thermal treatments such as

composting and thermal alkaline stabilization were also considered to be effective. Equivalent processes (as described in USEPA 1999) were also considered to be effective in attenuation of pathogenic bacteria. The consensus of the workgroup was that adequate data exists to show that these processes can reduce pathogenic bacteria to levels that would not constitute a public health risk.

The efficacy of Class B (PSRP) treatment processes was also considered. These processes include: Anaerobic digestion; Aerobic digestion; Lime stabilization; Composting (without Class A time/temperature regime); and Air drying.

It was acknowledged that each of these treatment processes might be effective in reducing the number of pathogenic bacteria in biosolids. However, it is unlikely that any of these processes could reduce the level of pathogenic bacteria to an extent where unrestricted use would not constitute a public health risk. To achieve such levels would require additional processing and/or natural attenuation. The site restrictions required for land application of Class B biosolids can be effective in attenuation of pathogenic bacteria, however, it was not clear that sufficient management controls are being used to minimize the risk of exposure to or transport of pathogenic bacteria.

Processing/Control Technologies Conclusions/Recommendations

Workgroup conclusions/recommendations regarding detection/analytical methods are as follows: 1) Treatment processes are available to produce Class A biosolids that should not have a significant public health risk with respect to bacterial pathogens. 2) Unrestricted use of biosolids produced by Class B treatment processes would likely pose a public health risk.

Prioritized List of Research Needs

While the following needs are listed from highest to lowest priority, many of them should be considered for concurrent action:

- Indicator/pathogen correlation. Use of indicator organisms to determine the effectiveness of treatment processes is reasonable. However, the utility of such a system is dependent upon the relationship between the indicator and specific pathogens. The relationship between indicator microorganisms and specific pathogens surviving treatment needs to be established.
- There appears to be limited data on the occurrence and survival of specific organisms such as enteropathogenic *E. coli, Shigella* and *Campylobacter*. Information on survival through treatment and in the environment is needed to determine a relative risk of exposure.
- Current methods for analysis of indicator bacteria and salmonellae do not appear in a standardized form, nor have these methods been evaluated by inter-laboratory testing. Standardization and validation of these methods needs to be completed.
- The adequacy of the site restrictions to land applied Class B biosolids needs to be evaluated with respect to protection of ground and surface water, vector transport, fugitive dust and aerosolization.
- With the high number of antibiotic resistant microorganisms being discharged into wastewaters, their fate with respect to biosolids treatment and management practices needs to be evaluated to ensure that organisms possessing resistance are not preferentially surviving treatment.
- Various management strategies and controls are in use for land application of biosolids. The effectiveness of these strategies in minimizing exposure needs to be evaluated.
- The effectiveness of Class B treatment processes in reducing the number of pathogenic bacteria needs to be determined.
- Evaluate worker safety (exposure) from wastewater treatment plant (WWTP) to application site.
- Evaluate the effectiveness of public access restrictions to land application sites.
- Evaluate the effectiveness of enforcement of biosolid regulations.
- Determine if the current monitoring frequencies are adequate to ensure the effectiveness of biosolids treatment processes.

- Evaluate the need for certification of laboratories conducting microbiological analysis of biosolids and similar by-products.

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Animal Manure: Bacterial Pathogens and Disinfection Technologies

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Introduction

The major concerns with emerging and re-emerging bacterial pathogens in animal manure are associated with the huge increase in foodborne illnesses in the U.S. over the past decade and the potential for contamination from manure to enter the food chain (Armstrong et al. 1996; Bettelheim 1996; CAST 1994) or water supplies. Commodities that are consumed raw are of special concern (Abdul-Raouf et al. 1993; Cieslak et al. 1993). Mead et al. (1999) estimated that more than 76 million cases of foodborne illness occur annually in the U.S., with 323,000 hospitalizations and 5,200 deaths. These large numbers for illness mean that substantial economic loss occurs in work time and effort. Presently, the portion of illnesses connected directly or indirectly to contamination of food at some point in the production, harvest, post-harvest, or preparation is not known. Furthermore, the quantitative impact that an effective program of manure treatment might have on reducing foodborne illnesses is unknown.

Livestock and poultry production in the U.S. supplies meat, poultry, dairy, and eggs for the American public and generates about $100 billion per year in farm revenue directly. This has a significant impact on people and economies of many rural communities. From 1982 to 1997, major structural changes in U.S. livestock and poultry production (Kellogg et al. 1997) have led to a decrease in the number of small farms (<150 animals) and a substantial increase in large farms (>300 animals). Although small farms still greatly outnumber large ones, they raise fewer animals. In 1982, farms producing more than 300 animals made up 3% of the total animal operations in the U.S. and they produced 35% of the total animal units (Kellogg et al., 1997). In contrast, by 1997, large farms made up nearly 5% of the total animal operations, but produced nearly 50% of the total animal units counted (Kellogg et al. 1997). Since confined animal operations provide an opportunity to collect and treat manures, they are a primary focus in this report. In 1982, farms raising more than 300 animal units in confinement contained 41% of the total confined animals. By 1997, they raised nearly 64% of confined animals. Still, confined animals as a percentage of total animals remained fairly constant at about 37% of the total raised in 1982 and 40% in 1997.

In 1999, the Government Accounting Office (GAO 1999) reported on a survey of animal operations in the U.S. The report described the type and extent of a variety of animal waste management practices available to livestock and poultry producers to limit runoff, and collect, store, treat, and use manures as fertilizer, additive to animal feed, or for on-farm energy generation. The practices were notably dependent on site-specific factors, costs, and state/local regulations. However, none systematically emphasized or prioritized control of zoonotic pathogens. In their report, the GAO (1999) noted that adaptation of multistage treatment technologies, such as those used for municipal wastewater, would require resolution of the economics involved in construction, maintenance and operation of more complex systems than are presently typical of livestock and poultry operations in the U.S.

For perspective, about 130 times more animal manure than municipal wastewater biosolids are produced in the U.S. annually. Some very large confined animal feeding operations (CAFOs), or areas with many aggregated CAFOs, produce as much raw manure as a town or a city produces treated sewage sludge, hereafter referred to as biosolids (GAO 1999). The concentration of such large volumes of raw manure in some areas can impact surface and groundwater quality — especially when spills, leaks, and/or runoff are not adequately managed — and ultimately impact the microbial quality of fresh produce. In response to these water and food impacts, new manure and nutrient management practices have been proposed and/or implemented in

various jurisdictions throughout the U.S. However, the focus of these new efforts has been primarily on nutrient management, or treatment/use technologies directed at nutrients. Pathogen reduction has been an auxilliary focus of the nutrient management strategies.

Presently, there are no regulations in place with the express purpose of preventing the transmission of pathogens from animal manure to freshly marketed produce. The U.S. Food and Drug Administration (FDA), which is responsible for safety of fresh produce (not meat, poultry, milk, or eggs), has published a set of guidelines for fruit and vegetable producers who use manures (in some form) in their cropping systems. These FDA recommendations are a series of common sense and good management practices (FDA 1998).

Concentration of Animal and Manure Production in the U.S.

As Table 1 shows, more than 300 million tons of manure were produced by beef and dairy cows raised in confined feeding operations in the U.S. in 1997 (Kellogg et al. 1997). Approximately 100,000 tons of swine manure were produced in 1997 in the U.S., with over 96% of it attributable to confined animals (Table 2). The quantity of poultry manure (chickens and turkeys) generated in 1997 was between 50 and 90 million tons. While swine and dairy manure typically are liquid slurry (manure plus urine with varying solids content, depending upon animal diet, bedding, and collection procedures), poultry broiler manure is usually quite dry because it is mixed with bedding and urine is not excreted. However, layer manure from battery cage operations, where no litter is used, has high moisture content. The type, amount, physical form and nutrient status of various manures affect the types of treatment processes that currently are used or are being developed for various types of animal operations.

While production of heifers, grass-fed animals, and calves comprises the greatest number of bovine animals (Kellogg et al. 1997), these animals are mainly raised unconfined, thus manure production from confined operations comprised less than 8% for this animal category in 1997. Since 1982, increases in the number of swine and poultry (which are mainly produced in CAFOs) are partially offset by decreases in the number of dairy animals raised; thus, the total number of confined animal units remained relatively stable, 35.4 million vs. 32 million between 1982 and 1997 (Table 2). Changes in consumer demand for livestock and poultry products in America and increased efficiency in the dairy industry between 1982 and1997 are reflected in the decreased numbers of all operations (Table 2); these changes impact the density and geographical distribution pattern of manure production.

Confined fattened cattle are mostly produced in the far midwest (Texas, Oklahoma, Nebraska) and the Northwest (Nevada, Oregon, Idaho and Washington). These are also the areas that have experienced the largest increases in beef CAFOs during 1982-1997. Dairy CAFOs are more evenly distributed throughout

Table 1. U.S. Bovine, Swine, and Poultry Manure Production for 1997

Animal (# animals/animal unit)	**# of AU**	**Tons manure/ AU/ yr**	**Total tons manure/yr***
Fattened Beef Cattle (1.14)	9,558,198	***10.59***	101,539,017 [98,679,658]
Producing Dairy Cows (0.74)	12,289,085	***15.24***	187,285,655 [150,846,563]
Other cattle: Heifers, breeders, grass-fed, calves (0.94 to 4.0)	58,787,447	~12.	705,449,364 [53,700,408]
Total Bovine	80,634,730	12. to 15.24	994,724,036 [303,226,629]
Breeding Hogs (2.67) and Hogs for Slaughter (9.09)	8,522,082	6.1 to 14.7	52,069,921 to 125,189,385
Chickens (250 – 455) and Turkey (50 - 67)	6,122,411	17.3 to 34.7**	50,081,322 to 91,652,493

*[ ] = amount of manure from confined animals.
**Total chicken = 14.97 (broiler) +11.45 (layer) + 8.32 (pullet), plus total turkey = 9.12 (breeder) + 8.18 (for slaughter), mostly all confined. Based on Kellogg, et al. 1997.

Table 2. Trends in American Animal Agriculture 1982-1997 in Number of Total And Confined Animal Units (AU) by Animal Category

Animal Category	# All Operations (x1000)	# Confined AU Operations (x1000)	Total # AU (Millions)	Total AU Manure (Million Tons/yr)**	Total # Confined AU (Millions)	Confined AU Manure (Millon Tons/yr)**
Beef	233/109* -53%	98/47 -52%	9.7/9.6 -1%	103/102 -1%	9.1/9.3 +2%	96/98 +2%
Dairy	271/115 -57%	161/86 -47%	14.7/12.3 -16%	223/187 -16%	11.3/9.9 -13%	172/150 -13%
Other Bovine	1,273/994 -22%	544/447 -18%	59.9/58.8 -2%	719/706 -2%	4.7/4.5 -5%	56/53 -5%
Swine	317/103 -67%	175/63 -64%	7.3/8.5 +16%	45-107/51-124.95 +16%	6.3/8.2 +31%	38-92/52-125 +31%
Poultry	196/75 -62%	66/35 -46%	4.0/6.1 +52%	69-139/106-212 +52%	4.0/6.1 +52%	69-139/105-559 +52%
Totals	2290/1396	1044/678	95.6/95.3	1159-1291/1152-1332	35.4/32	431-555/458-985

*All cells: 1982 value / 1997 value and % change; Based on Kellogg, et al. 1997.**Based on Tons manure/AU-year: Beef, 10.6; Dairy 15.2; Other Bovine, 12; Swine, 6.1 to 14.7; Poultry, 17.3 to 34.7

the U.S., but they have increased in the southwest and Alaska during 1982-1987. Cattle in the 'other' (heifers, breeders and calves) category are also evenly distributed, with significant increases noted especially in the far midwest and California. Swine production is concentrated in the midwest (generally around Iowa), the Carolinas, and Arizona with large increases during 1982-1997 in North Carolina and Arizona. Poultry production is more heterogeneously distributed, with major production concentrated along the Eastern seaboard, the Gulf Coast, and California. The Delmarva Peninsula, Carolinas, and California have experienced large increases during 1982-1997. The various practices available to livestock and poultry producers for handling and storage of animal manure are described in part in the National Handbook of Conservation Practices (USDA/NRCS 4/26/99; also on the internet at http://www.ftw.nrcs.usda.gov/nhcp_2.html). However, these are primarily engineering design standards and do not typically address routine operation issues or pathogen destruction specifically.

The information presented here focuses on relevant aspects of the major bacterial pathogens of concern in animal manures relative to food safety and water quality. Recent outbreaks of zoonotic diseases at special events where infected farm animals and associated manure were present constitute notable special situations that producers and specialists should be informed about (Reintjes et al. 2000). In addition, it presents information about what is known about the effects of common treatment technologies on the survival of these pathogens. Detection and enumeration are integral components of any programmatic assessment of the source loads, fate and transports of these bacterial pathogens. Thus, attention is also focused on information and availability of rapid detection methods, especially those that may enable rapid, specific detection and enumeration/quantitation of viable pathogens.

Emerging and Re-Emerging Bacterial Pathogens

Most of the bacterial pathogens of concern in animal manures have been around for a long time. Many have been the focus of considerable study by veterinary, public health, and sanitation scientists as well as agricultural, municipal and international organizations (Feachem et al. 1983; Haapapuro and Barnard 1997; Pell 1997; Strauch and Ballerini 1994; Tauxe 1997). Among the many potential pathogens, a few have featured prominently in agricultural and public health sectors for the past two decades because of their implication in the increasing incidence of foodborne illness outbreaks (Ackers et al. 1998; Altekruse et al. 1998; Brackett 1999; Hilborn et al. 1999; Roberts et al. 1995; Strauch and Ballerini 1994). The descriptions that follow highlight some of the major bacterial pathogens that are of primary concern relative to manure use in agriculture and their impact on food safety and water quality: enterohemorrhagic *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Mycobacterium avium* subsp. *paratuberculosis*, *Yersinis enterocolitica*, *Clostridium perfringens*, *Bacillus cereus*, *Enterococcus,* and antibiotic resistance.

A variety of methods have been used in different laboratories to detect and quantify bacterial pathogens in manure, soils, and water. At this time, however, none of the methods are validated as standard methods for manure. Hence, it is somewhat difficult to make precise comparisons between the values reported for prevalence and survival of microorganisms from different studies. In some cases, results from different locations appear inconsistent. The increased use in clinical and food microbiology of rapid, molecularly-based detection methods has led to development of some universal polymerase chain reaction (PCR) protocols (Bach et al. 2002; Greisen et al. 1994; McCabe et al. 1999; Wang et al. 1997) possibly applicable to the detection of many of the organisms described here for manure. Examples, rather than a comprehensive list of rapid methods for each organism or groups of organisms, are provided in the sections that follow below. However, specific tests and validation studies of these and other protocols are needed for consistent results that will be useful in comparisons of treatment technologies, where spiked samples or other quality control and assurance procedures differ between assay methods.

Enterohemorrhagic *Escherichia coli*

Many, but not all, cases of *E. coli* O157:H7 infection have been associated with consumption of contaminated hamburger from feedlot beef cattle or old or culled dairy cows. This organism is known to inhabit the gut of mature cows without causing any illness, and also establishes in calves and other animals (Faith et al. 1996; Besser et al. 1993; Beutin et al. 1993, 1995; Hancock et al. 1994; Mechie et al. 1997; Porter et al. 1997; Willshaw et al. 1993; Zhao et al. 1995). Several investigators have examined the hypothesis that various stressors, e.g., fasting, diet, slaughter (Dargatz et al. 1997; Diaz-Gonzales et al. 1998; Fedorka-Cray et al. 1998; Garber et al. 1995; Hovde et al. 1999; Wallace et al. 1989), are important determinants in the sporadic increased shedding by cows, steers, and calves. The intermittent rather than continuous shedding has also contributed to the difficulty in assessing prevalence of these strains in herds. Despite reports such as Harmon et al. (1999) and Kudva et al. (1997) that show little effect on fecal shedding and rumen proliferation of *E. coli* O157:H7 when feed is withdrawn, the topic remains an active area of research (Arthur et al. 2001; Diaz-Gonzales et al. 1998). Tkalcic et al. (2000) showed that rapid development of acid tolerance by *E. coli* O157:H7 in the rumen of calves fed high-concentrate diets may be an important factor that influences fecal shedding in cattle.

Recent molecular genetic analyses of enterohemorrhagic *E. coli* (EHEC), particularly O157:H7, and other shiga-toxin (*stx*) producing, and non-enterohemorrhagic *E. coli* strains (nearly 1400 genes differ between O157:H7 and *E. coli* K12, Perna, et al. 2001), present an interesting study in the evolution of a highly pathogenic sub-group of an ordinarily harmless bacterium (Whittam et al. 1993). The O157:H7 ancestor carried the EHEC locus of enterocyte effacement (LEE) pathogenicity island and acquired the *stx* genes from lysogenic *Stx* phage (Feng et al. 1998) as well as plasmid-borne pathogenicity factors to become the primary cause of hemolytic uremic syndrome in the US. However, the enterohemorrhagic strains were just recognized as a distinct group of *E. coli* in 1982 so their impact on man is a relatively recent event. The EHEC and *stx* strains have especially severe disease consequences among very young, very old, and immuno-compromised people, in contrast to the lesser consequences among strong, healthy people in the general population (Griffin and Tauxe 1991). The EHEC and *stx* strains can have a very low infectious dose; some estimates show as few as 10 or 100 cells for certain strains will cause disease in a susceptible individual, according to analyses presented by Powell et al. (2000). Although EHEC share many pathogenicity genes with other pathogenic forms of *E. coli*, they have a unique combination of adherence factors, toxins, and hemolysins that make them particularly virulent and devastating when a susceptible person becomes infected.

During 1991–1992, a national survey of *E. coli* isolates from 1,305 (of 6,894) fecal samples was conducted by USDA, Animal and Plant Health Inspection Service, National Health Monitoring System, Dairy Heifer Evaluation Project. The isolates were tested for virulence attributes known to be associated with human EHEC as well as for the enterotoxin commonly associated with diarrhea in newborn calves (Cray et al. 1996). Single, random isolates from each heifer were hybridized to probes derived from the 60 mDa EHEC plasmid (CVD 419), *eae*, Shiga-like toxin (SLT) genes I and II, and *E. coli* heat-stable enterotoxin a (STaP). Seventy-seven of the 1,305 isolates (5.9%) were found to be SLT-positive. Most (81.8%) SLT-positive *E. coli* were also CVD 419 and *eae*-positive. Only 2 of the SLT-positive *E. coli* isolates were STaP-positive.

Subsequently, Beutin et al. (1995) showed that shiga-like toxin (verotoxin)-producing strains of *E. coli* (SLTEC) originating from healthy cattle, sheep, goats, swine, cats, and dogs were a very heterogeneous group of *E. coli*. Many of these strains appeared to be specific for their hosts. The absence of *eae* sequences (i.e., *E. coli* attaching and effacing genes) in most of these animal SLTECs may mean that these strains are less virulent for humans than the classical *eae*-positive EHEC strains. For the 29% SLTEC-positive healthy animals (208 of 720), Beutin et al. (1993) reported 67% frequencies of SLTEC carriers in sheep, 52% in goats,

and 21% in cattle. Frequencies of SLTEC strains for other animals examined was 7.5% in swine, 14% in cats, and 5% in dogs, and less than 0.7% (undetectable) in chickens. In a separate study, Kim et al. (1999) showed that bovine EHEC isolates comprised only one-third of the more virulent group of two EHEC/*stx* clades, and that very few human isolates clustered in the low virulence, bovine clade.

In addition to pathogenicity genes, *E. coli* O157:H7 also has acquired genes that may encode alternative metabolic capacities or confer extraordinary resistance to environmental conditions (Conner and Kotrola 1995; Diaz-Gonzales et al. 1998; Valcour et al. 2002; Wang and Doyle 1998b), which endow it with an increased tolerance to pH shifts among other things. This may mean it will not behave like other *E. coli* in all situations.

Detection, Quantification, and Source Tracking

Enterohemorrhagic strains of *E. coli* sometimes have been difficult to recover from environmental samples because of their sporadic seasonal occurrence patterns, sometimes low densities, and previous low sensitivity detection methods (media not selective enough). Methods for detection, quantification, and, importantly, source tracking are continually being improved and adapted. Hence, precise methodological consistency between different laboratories remains an issue, although most methods share common features. Immunomagnetic bead separation (IMS) and enrichment techniques followed by selection of sorbitol negative colonies and confirmatory tests currently are used for specific recovery and quantitation of viable cells, even with bovine fecal samples (Chapman et al. 1994; Parham et al. 2003). In a recent report, Robinson et al. (2004) show that spiral plating of bovine fecal samples can be an efficient and accurate approach for epidemiological survey studies in which the lowest detectable level is 100 cfu/g and an increasingly greater variation in counts observed at low cell densities. Other reports cite use of a variety of methods for isolation, identification, and quantification of solid and liquid samples (Beutin et al. 1993; Higgins et al. 2001; LeJeune et al. 2001, 2001a, 2001b; Maurer et al. 1999; Schmidt et al. 1995; Shelton and Karns 2001; Shelton et al. 2003, 2004; Wang et al. 1996, 1997). Campbell et al. (2001) reported the successful use of a two-stage enrichment followed by multiplex PCR for detection of *E. coli* O157:H7 at low sensitivities: 1 cfu/ml in drinking water and 2 cfu/g soil. Using 8-hr primary enrichment, they detected 6 cfu/g soil. Real-time PCR (Ibekwe et al. 2002; Sharma et al. 1999) has been developed for rapid detection, and RT-PCR (Yaron and Matthews 2002) and nucleic acid sequence-based amplification (NASBA) hold promise for assessing viability quickly. With real-time PCR assays for eae and stx genes, Sharma (2002) was able to detect as few as 1 to 10 *E. coli* CFU/g of feces from an 18 hr enrichment. Comparable sensitivity, i.e. 1 CFU/g, was achieved by Szabo et al. (1990) using a hydrophobic grid membrane filter enzyme-labeled antibody technique for *E. coli* O157. Cost-effective, routine molecular enumeration has yet to be achieved for manure, manured soils, and composts. For source tracking of *E. coli*, Guan et al. (2002) showed that amplified fragment length polymorphism was the most effective method compared with 16S sequencing and multi-antibiotic resistance patterning for differentiating *E. coli* isolates of livestock, wildlife, or human sources in polluted water.

Survival

Lau and Ingham (2001) reported that *E. coli* and enterococci can survive at least 19 weeks at 9-21°C in silty clay loam and loamy sand soils amended with fresh bovine manure. Ogden et al. (2001) reported that *E. coli* O157 survived 15 weeks in a loamy sand soil on which contaminated sheep feces were naturally deposited. An immunomagnetic separation post-enrichment was used along with plating onto sorbitol MacConkey agar and 3-tube MPN. Gagliardi and Karns (2000, 2002) showed that roots and different soils influence survival (up to 86 da); and leaching of *E. coli* in microcosm experiments and the manure itself does not protect or aid survival (in controlled environment chambers). Fukushima et al. (1999) found that shiga toxin-producing *E. coli* O26, O111, and O157 survival ranged from 1 to 18 weeks at 15ºC . Jiang et al. (2002) also recently reported that *E. coli* O157:H7 viability declined more rapidly in field soil with and without manure (10%) incubated at 15 and 21ºC, than in autoclaved soils in which it survived for 77, >226, 231 da at 5, 15, and 21ºC incubation temperatures.

Water has been clearly shown to play a major role in the establishment and persistence of various strains of *E. coli* O157:H7 in herds (LeJeune et al., 2001 a, b). Preventing manure-contaminated water from running off into surface waters are a part of many nutrient control efforts and could reduce pathogen transmission and subsequent survival (Baxter-Potter and Gilliland 1988; Chapman 1996; Chaubey et al. 1994; Coyne 1995; Coyne et al. 1994; Doyle et al. 1975; Giddons and Barnett 1980; Howell et al. 1995; Mawdsley et al. 1995; McCoy and Hagedorn 1979; Quisenberry et al. 1981). Runoff and other direct forms of contamination can recharge surviving and viable but nonculturable (VBNC) states of microbes including *E. coli* (Miettinen et al. 1997). For some strains in surface waters, this could increase the possibility of subsequent

consumption by animals. This scenario has in part been the subject of a study (Wang and Doyle 1998a) examining survival of *E. coli* O157:H7, in which results showed that this strain can survive for a long time in water, especially at cold temperatures, and at 25 ºC for 12 weeks remained viable in direct viable count assays, but undetectable on tryptic soy agar plates; this indicates that *E. coli* O157:H7 may enter a VBNC state in water.

Salmonella

*Salmonella*e are a group of bacteria known to be pathogenic to humans (gasteroenteritis, diarrhea, possible septicemia) and animals (diarrhea, septicemia) that are transmitted by the fecal-oral route — through consumption of contaminated food or water. There are many different serovars of *Salmonella* and some seem to have specific host ranges. The difficulty in distinguishing serovars makes study of prevalence of specific *Salmonella*e serovars difficult. Development of rapid methods of identification combined with quantification are required. Forms that are pathogenic to man are known to be associated with poultry products (eggs and meat), pork, beef, and milk. However, except for eggs, it is often uncertain whether the strains responsible for foodborne outbreaks are associated with the food consumed or with the food handler (Oosterom 1991). Emergence of multiple antibiotic resistant *Salmonella*e, such as *S. enterica* serovar. *Typhimurium* DT104, have added a critical new concern about the transmission of this group of pathogens from animal production operations to other animals and humans (Evans and Davies 1996; Khachatourians 1998). This concern led to the establishment in 1996 of the National Antimicrobial Monitoring System, a joint effort between the Centers for Disease Control and Prevention, the U. S. Department of Agriculture, and the Food and Drug Administration. This program prospectively monitors changes in antimicrobial susceptibilities of zoonotic pathogens from human and animal clinical specimens, from healthy farm animals, and from carcasses of food-producing animals at slaughter (Tollefson et al. 1998). Recently, Leon-Velarde et al. (2004) using PCR and antibiotic resistance assays reported a prevalence rate of 5.5% for *Salmonella enterica* serovar. *Typhimurium* DT104 were among 435 *Salmonella* isolates over a 20 month study period in commercial poultry houses studied.

Salmonella infection of animals often causes losses to the farmer, so its persistence and dissemination on-farm has implications beyond the possibility of product contamination (Wray 1975). Davies et al. (1997) reported increased shedding of *Salmonella* spp. by finishing-age swine housed in barns with open-flush gutters in comparison with slotted-floor barns, possibly because repeated exposure to infected feces prolongs fecal shedding by swine. In the 1970s and 1980s, studies on *Salmonella*e survival were conducted in connection with farm applications of manure and sewage, and evidence then showed that *Salmonella*e could survive for a limited time as a result of land application, but not as long as other pathogens or parasites (Giddons and Barnett 1980; Jones et al. 1976; McCoy and Hagedorn 1979; Rankin and Taylor 1969; Snowdon et al. 1989; Thunegard 1975; Will et al. 1973; Wray 1975).

In a national study of the health and management of feedlot cattle (Fedorka-Cray et al. 1998), *Salmonella*e were recovered from 38% (38 of 100) of the feedlots. *Salmonella enterica* (*S.e.*) serovars were recovered from 5.5% (273 of 4,977) of all samples and from 3.5% (88 of 2,484) and 7.4% (185 of 2,495) of samples from pens of cattle on feed for short and long time periods, respectively. The most common serotype recovered was *S.e. anatum* (27.9%), followed by *S.e. montevideo* (12.9%), *S.e. muenster* (11.8%), *S.e. kentucky* (8.2%), and *S.e. newington* (4.3%). The most common serogroups identified were E1 (39.6%), C1 (20.7%), and B (10.4%). Shedding of serotypes most commonly associated with human illness occurred infrequently (13 of 273: 4.8%). Most isolates were not resistant to any antimicrobial tested.

Detection and Quantification

In a comparative study of methods for detecting *Salmonella* presence from dairy cattle, poultry, and swine, Panglioli et al. (2003) reported that the effectiveness of recovery from pre-enrichment in lactose broth vs. direct enrichment prior to selective enrichment in Rappaport-Vassiliadis, selenite cystine, and tetrathionate incubated at 35 and 42ºC and in four differential/selective plating media (brilliant green, bismuth sulfite, Hektoen enteric, and xylose-lysine-tergitol 4 agar) depended on the type of sample. Thus, no generalized media would be equivalently effective for all animal and farm source samples. However, using a molecular approach Simpkins et al. (2000) reported the successful use of an rRNA method, NASBA, to detect viable cells of *S. enterica*. Burtscher et al. (1999) reported a method for extraction of nucleic acids from organic wastes and subsequent PCR detection of *Salmonella* and *L. monocytogenes*. Ziemer and Steadman (2003) found three primer pairs that were useful for PCR amplification of *Salmonella enterica* in faecal samples – 16S rDNA, stn enterotoxin gene, and histidine transport operon. Civilini et al. (2000) reported on an improved IMS-PCR method for *S.e. Typhimurium* in compost enabling detection of 30 cfu /50 g compost by 30 hr.

Campylobacter

Campylobacter jejuni and *C. coli* are primarily associated with poultry and swine, and to a lesser extent sheep and cattle, although they do not typically have adverse effects on these hosts. In humans, *Campylobacter* spp. are the most common bacterial cause of gasteroenteritis in the U.S. The symptoms of *Campylobacter*ial infection are similar to those of appendicitis, and consequently infection in humans sometimes has led to unnecessary surgery. In addition to being one of the foremost causes of gasteroenteritis, *Campylobacter* is also one of the bacteria in which resistance to antibiotics, especially quinolones, has occurred in humans who consumed poultry. After use of this group of antibiotics was approved for use in poultry in Europe (Endtz 1991), the prevalence of enrofloxacin-resistant strains of *Campylobacter* in poultry and humans increased from 0–14% and from 0–11% respectively. Investigations in poultry showed that it is not transmitted from breeder flocks via hatchery to progeny, nor from one flock to the next in a broiler house. The major route of *Campylobacter* transmission to poultry is horizontally from elsewhere in the environment. Furthermore, a large number (29%) of isolates in one study of broiler flocks were resistant to the antibiotic quinolone (Jacobs-Reitsma 1997). They apparently inhabit the intestine of poultry causing no ill affects. *C. jejuni* is responsible for most human cases of infection. They have occasionally been associated with beef and pork. Aluminum sulfate (alum) and sodium bisulfate have been used as a poultry litter treatment to reduce volatile ammonia in poultry houses. Line (2002) reported that alum (3.63 or 7.26 kg/4.6 m^2) and sodium bisulfate (1.13 or 1.81 kg/4.6 m^2) significantly ($P < 0.05$) reduced *Campylobacter* colonization frequency and populations in the ceca. Thus, he concluded that an acidifying litter treatment in poultry houses may help control *Campylobacter* and reduce its horizontal transmission in broiler flocks.

Detection and Quantification

Sails et al. (1998) reported the development of a RT-PCR assay for detection of viable *Campylobacter* spp., whereas previously Uyttendaele et al. (1995) reported the use of NASBA for assessing viable *C. jejuni.* Van Doorn et al. (1999) reported the use of a GTPase-based PCR reverse hybridization assay for *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* from various geographic locations. Nogva et al. (2000) reported development of a rapid, highly sensitive PCR for *C. jejuni* that was quantitative over 6 logs. Neither method has been adapted to manure and water samples. However, Itoh et al. (1995) reported the use of PCR for detection of *C. jejuni* in chicken litter. Wesley et al. (2000) reported development of a multiplex-PCR method and successful detection of *Campylobacter jejuni*, *Campylobacter coli*, and *Arcobacter* spp. from dairy feces. Line (2001) reported the development of a selective differential agar for isolation and enumeration of *Campylobacter* spp., and subsequently developed a selective broth by which he was able to detect stressed *Campylobacter* growth using capacitance monitoring (Line and Pearson, 2003).

Listeria monocytogenes

Listeria monocytogenes is a particularly interesting organism because it is ubiquitous in nature, and sometimes present in high numbers in soil, rotting vegetation (including silage), water, and feces (Dowe et al. 1997; Iida et al. 1998; Van Renterghem et al. 1991). Nesbakken et al. (1996) reported that the *Listeria monocytogenes* strains found in food and in human cases are, for the most part, different from those entering the front door of the slaughter plant with live animals. However, rapid and specific isolation and detection methods for *L. monocytogenes* that are currently applicable to human and food samples need to be modified and validated for use with environmental samples, especially manure, soils, and runoff water. Methods to rapidly and conveniently isolate pathogenic forms from a range of environmental sources and manures also are needed in order to compare the efficacy of different manure treatment processes. The use of PCR, fluorogenic probes, and chromogenic substrates for detection of *Listeria monocytogenes* (Bassler et al. 1995; Restaino et al. 1999) will aid in future ecological studies on the survival and distribution of this organism on farms and in the environment.

The U.S. Centers for Disease Control estimates there are 2,500 cases of listeriosis in the U.S. each year with 500 of them fatal and strongly associated with infants, children, senior citizens, or those individuals with impaired immunity. Certain serotypes are associated with most human infections and methods for their detection and typing have been developed (Iida et al. 1998; Jersek et al. 1999; Manzano et al. 1997; 1998; Nannapeneni et al. 1998). In addition, isolates reportedly harbor plasmids that confer resistance to antibiotics (Lemaitre et al. 1998), and therefore have some capacity to overcome existing clinically therapeutic approaches. In addition to human impacts from *Listeria*, significant sporadic economic losses, from abortion, occur among farm animals in the U.S. Hence closing the gap on disease transmission on-farm has potential direct benefit to producers.

Detection and Quantification

Klein and Juneja (1997) reported that an RT-PCR assay was a sensitive method for detection of viable *L. monocytogenes*. Using organic waste matter, Burtscher et al. (1999) reported a method of nucleic acid ex-

traction and PCR detection of *L. monocytogenes* and *Salmonella*. Focusing on the *iap* gene encoding the p60 protein, Bubert et al. (1999) were able to develop a multiplex PCR for identification of *Listeria* species and strains from a wide range of sources. Subsequently, to overcome the intense competition that occurs when culturing for *Listeria* on differential agar media, Bauwens et al. (2003) combined the use of immunomagnetic separation with a chromogenic isolation medium; this resulted in improved detection of pathogenic *Listeria* spp. in a variety of zoo animal feces.

Mycobacterium avium subsp. paratuberculosis

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease in cattle (Rossiter and Burhans 1996; Whittington and Sergeant 2001). This organism was suspected of an association with human intestinal disease, but recent forensic DNA evidence (Baksh et al. 2004) fails to support this concept. A fecal-oral route is the main pathway of disease transmission (Wray 1975). Recent reports suggest that cattle grazing in fields with infected rabbits may play a role in on-farm transmission of the disease (Daniels et al. 2001). Nematodes (Whittington et al. 2001) as well as larval and adult blowflies (Fisher et al. 2004) may vector MAP. Other factors found to be important in determining the likelihood of individual dairy cow infections include the annual importation rate, herd size, and presence of other MAP seropositive individuals in a herd (Hirst et al. 2004). The very slow rate of growth of MAP on selective media has made it a difficult bacterium to study. Stehman et al. (1996) reported that MAP survived up to 252 days at 15 °C and 98 days at 4 °C in cattle manure slurry; up to one year in soil; and 113–270 days in inoculated pond water. Whittington et al., (2004) reported MAP survival for up to 24 weeks on grass that germinated through infected manure applied to the soil surface in completely shaded boxes and for up to 9 weeks on grass in 70% shade, but up to 55 weeks in a dry 100% shaded site. In general, this bacterium has exceptional heat tolerance, but it does succumb to pasteurization temperatures. However, in addition to being recovered from some bulk milk tanks, it has also been recovered from pasteurized milk samples in the UK (Grant et al. 2002a, 2002b). Klijn et al. (2001) reviewed experimental conditions of the MAP heat inactivation studies of different research groups and found significant variations that led to considerable differences in results and conclusions. Klijn et al. (2001) suggest that a more collaborative and standardized research approach should be employed in future studies involving multiple laboratories and locations.

Detection and Quantification

Mason et al. (2001) compare the use of IMS-PCR with direct culture of MAP from sheep fecal specimens. Fang et al. (2002) reported high sensitivity (1 to 8 cfu) and specificity (92%) for detection of MAP with automated PCR using fluorescent probes (molecular beacons) for bovine feces. A real-time NASBA system for detection for MAP also has been developed recently and was reported to have a sensitivity of 150-200 cells per reaction (Rodriguez-Lazaro et al., 2004), but it has yet to be applied to manure.

Yersinia enterocolitica

The genus *Yersinia* has three species: *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. All can be pathogenic to humans and animals. *Yersinia enterocolitica* is associated with swine sporadically (Anderson et al. 1991; Morris and Feeley 1979) and with wildlife. The incidence of gastroenteritis caused by this bacterium is rising, possibly because of increased awareness and detection. Also, it can cause pseudoappendicitis. Nonpathogenic strains of this bacterium survive outside animal hosts, and survive in stored manure slurry for 12 and 21 days at 17°C and 4°C , respectively (Kearney et al. 1993a).

Advances in rapid detection methods for *Y. enterocolitica* using sensitive fluorogenic TaqMan-PCR (Vishnubhatla et al. 2001) and other probes (Trebesius et al. 1998) have been reported. They have not yet been adapted to manure or runoff/ground water samples. Trebesius et al. (1998) used their probes to detect *Y. pestis* and *Y. pseudotuberculosis* in throat and stool samples, and *Y. enterocolitica* in tissue samples.

Clostridium and Bacillus

Clostridium perfringens type A food poisoning is one of the most common foodborne illnesses in the industrial world (Brynestad and Granum 2002). However, because of the mildness and self-limiting nature of the illness, many cases are undiagnosed. *Bacillus cereus* is a ubiquitous, gram-positive bacterium that causes a significant number of foodborne illnesses. *Clostridium perfringens* is a common resident of the intestines of animals, where it causes gastrointestinal and enterotoxemic diseases. *Clostridium perfringens* can cause gasteroenteritis in humans through the production of a toxin which is stimulated by its own production of spores. *Bacillus cereus* is an important food spoilage organism widely found in soil and isolated from fruits, vegetables, nuts, milk, meat, dried food and spices (Kramer and Gilbert 1989). In contrast to the other pathogenic bacteria described above, both *Clostridium* and *Bacillus* species form spores that are especially resistant to heating and drying. This has implications for long-term survival and dissemination in soil,

water, and air because spores can readily survive significantly greater stressors than do vegetative bacterial forms. Because of their resilience due to their ability to produce endospores, *C. perfringens* and *Bacillus* can persist and survive many manure treatment technologies. Despite their resilience, Juteau et al. (2004) reported that substantial reduction in numbers of *C. perfringens* was obtained by operating a 59-L aerobic thermophilic sequencing batch reactor at 60°C with a 6 da retention time. In addition to its importance as a pathogen and a sulfite-reducer, *C. perfringens* has also been suggested as a conservative indicator of fecal contamination in air and estuarine, ocean, surface/groundwater.

Detection and Quantification

Anaerobic growth and formation of black colonies on typtose sulfite cycloserine agar in 24 hr is presumptive evidence of *C. perfringens*. Standard methods of confirmation take about 72 hr, whereas the new chromo-fluorogenic media (containing o-nitrophyl-β-D-galactopyranoside, ONPG, and 4-methylumbelliferyl phosphate, MUP) described by Adcock and Saint (2001) reduces the test to 4 hr. Recent molecular technologies have advanced the capacity to identify *C. perfringens* and *B. cereus* relative to closely related species (Augustynowicz et al. 2002; Petit et al. 1999; te Giffel et al. 1997). A multiplex PCR useful for genotyping toxin producing strains obtained from a variety of animal sources is also available (Baums et al. 2004). Recently, a microarray chip capable of identifying eight toxin producing strains of *C. perfringens* has been reported (Al-Khaldi et al. 2004). However, these molecular methods have yet to be adapted to manure, wastewater, slurry, or manured soil.

Enterococcus and Antibiotic Resistance

The widely distributed genus *Enterococcus* comprise a group of hardy, gram-positive diplococci of fecal origin that are morphologically, biologically, genetically, and serologically distinct from the genus *Streptococcus*. Five species groups of *Enterococcus* have been determined by 16S rRNA sequencing: *faecium, avium, gallinarum, cecorum*, and other distinct species (which includes *faecalis*). One species in particular, *E. faecium*, was designated the nocosomial pathogen of the 1990s (Spera and Farber 1992) because of its intrinsic resistance to many antibiotics and its propensity to acquire resistance to the majority of clinically useful antibiotics (particularly vancomycin). In addition, there is evidence that vancomycin and other resistance genes are highly mobile via broad host-range plasmids from *E. faecium* and *E. faecalis* to other species of the genus and other genera (Aarestrup et al. 1996; Huycke et al. 1998; Morrison et al. 1997). Discriminant analysis of antibiotic resistance patterns of *Enterococcus* has proven useful in source tracking fecal pollution in water (Wiggins 1996).

Levy et al. (1976) were among the first to study the connection between feeding farm animals antibiotics in rations and acquisition of antibiotic resistance in the intestinal bacterial flora of farm inhabitants and their neighbors. They found that when chickens were fed tetracycline-supplemented feed, their intestinal flora contained almost entirely tetracycline-resistant organisms in the week following start-up of antibiotics. Increased numbers of resistant intestinal bacteria also appeared, but more slowly, in farm members, but not their neighbors. Within five and six months, 31.3% of weekly fecal samples from farm dwellers contained greater than 80% tetracycline-resistant bacteria as compared to 6.8% of the samples from the neighbors.

Perhaps one of the most striking examples of the potential hazards associated with propagation of antibiotic resistance among *Enterococcus* spp. is their use as starter and probiotic cultures in foods, and their natural presence as food contaminants. In a study of the incidence of known virulence determinants in starter, food, and medical strains of *Enterococcus faecalis, E. faecium*, and *E. durans*, Eaton and Gasson (2001) found that medical *E. faecalis* strains had more virulence determinants than did food strains, which, in turn, had more than did starter strains. Further tests revealed that the starter strains acquired virulence determinants by natural conjugation with medical strains, and both food and starter strains have multiple genes that support plasmid acquisition.

Treatment Technologies — Why Treat Manure to Reduce Pathogens?

There are a number of reasons for encouraging producers to treat animal manure before applying it to fields as fertilizer or before selling it as a value-added product. For farmers to coexist with the general public and do their part as environmental stewards, manure management must include some type of treatment that will stabilize nutrients, reduce pathogens, control odors, and reduce the attraction of vectors. Control of pathogens and vector attraction (rodents, insects, and others) through a series of farm and manure management practices will benefit farm operators and animal producers in terms of improved animal health. Nutrient stabilization will improve nutrient conservation and odor control will improve community satisfaction with neighboring animal operations.

Another issue is the possible role of animal agriculture in the spread of antibiotic resistance determinants among bacterial populations; manure treatment that kills pathogens would reduce the possibility of such transfers. Antibiotic resistance in fecal bacteria of animals treated prophylactically with the same antibiotic has been increasingly reported (Aarestrup et al. 1996; Coque et al. 1996; van den Bogaard et al. 1997; Wegener et al. 1999).

The potential for contamination of water from manure pathogens is clearly greatest when untreated manure is surface applied to pasture, cropland, or other fields without regard to protective and diversion barriers, especially if a significant rainfall event occurs that results in runoff from a fresh field application. A USGS survey in the 1980s (Smith et al. 1993) indicated that high fecal coliform counts in stream water at various points across the US tended to be clustered in areas that had high intensity animal agriculture. In this survey, no attempt was made to identify the actual sources of the bacteria, but it is quite possible that animal manures were a source in some instances. Appropriate treatment of manure from CAFOs would virtually eliminate major potential releases of pathogens into U.S. waterways, especially during major storm events.

Treatment can render manure less attractive to birds, rodents, and insects that can vector pathogens. With fewer pathogens present, and less appeal to vectors, treated manure, even when stored, can provide improved control of pathogen dissemination within, around, and off the farm or animal production facility.

Types of Treatments Currently Used on Farms

Digestion and Lagoons

Use of anaerobic, mesophilic or thermophilic digesters is somewhat limited on farms in the U.S. at the present time, primarily because large capital expenditures are required to purchase the necessary equipment. However, lagoons and slurry tanks have functioned as typical anaerobic treatment processes for swine and dairy manure in many regions. They are occasionally used for high moisture content layer hen manure as well.

Temperature is an important determinant in survival of *Salmonella*e, as well as other bacteria, in slurry (Stehman et al. 1996). Kearney et al. (1993a) reported that viable numbers of *E. coli, S.e.* serovar. *Typhimurium, Y. enterocolitica,* and *L. monocytogenes* in beef manure slurry decline more rapidly at 17°C than at 4°C, and that mesophilic anaerobic digestion produced an initial rapid decrease in viable numbers followed by a period in which viable numbers remained relatively stable. The T90 values, i.e., time in days for 90% population reductions to be achieved, for *E. coli, S.e. Typhimurium* and *Y. enterocolitica* ranged from 0.7 to 0.9 days during batch digestion and 1.1 to 2.5 days during semi-continuous digestion. Strauch (1991) reported that *Salmonella*e survived in cattle slurry from 49-230 days at pH 7.0-7.7, but considerably less when pH was increased, i.e., 12-29 days, pH 9.0-9.4. Survival in swine slurry and poultry litter were 39-47 and 8-57 days at pH of 7.5-8.0.

In the case of stored poultry manure, Himathongkham et al. (2000) showed that populations of both *S.e. Typhimurium* and *E. coli* O157:H7 decreased log-linearly with time and temperature; T90 ranged from 12 h at 37°C to 1-2 wk at 4°C. With poultry manure slurry (2:1, water:manure), populations decreased more slowly; T90 ranged from 24-48 h at 37°C to 6-22 wk at 4°C.

Under aerobic-thermophilic (50°C) conditions, Herold et al. (1999) reported that *S.e. Typhimurium* DT104 and *E. coli* were significantly reduced in swine manure slurry in 3 hr. However, without this high temperature, populations declined only when microbial substrates were significantly reduced. Furthermore, pH fluctuations had no significant effect on these populations at mesophilic or thermophilic temperatures. In their studies of thermophilic anaerobic digestion of liquid manures, Soldierer and Strauch (1991) found that the combined time-temperature exposures for *Salmonella*e inactivation should not fall below the following values (assuming process temperatures are uniform throughout the digester): 50°C/15 h, 55°C/3 h, 60°C/30 min, 65°C/5 min.

Listeria monocytogenes had a significantly higher mean T90 value during semi-continuous digestion (35.7 days) than batch digestion (12.3 days). Subsequently, Kearney et al. (1993b) reported that numbers of *E. coli, S.e. Typhimurium, Y. enterocolitica, L. monocytogenes* and *C. jejuni* were reduced during full-scale mesophilic anaerobic digestion of beef manure slurry (operated at 28°C, daily fed, and mean hydraulic retention time of 24 days). *E. coli* had the smallest mean viable numbers at each stage of the digestion process and a mean T90 value of 76.9 days. *Yersinia enterocolitica* was the least resistant to this digester; its

mean T90 value was 18.2 days. *Campylobacter jejuni* was the most resistant bacterium; its mean T90 value was 438.6 days. Regression analysis showed that there were no direct relationships between the slurry input and performance of the digester and the decline of pathogen numbers during the 140 day experimental period.

Drying

Himathongkham and Riemann (1999) reported that drying poultry manure to a moisture content of 10% followed by exposure to ammonia gas in an amount of 1% of the manure wet weight reduced numbers of *S.e. Typhimurium* and *E. coli* O157:H7 by 8 $\log_{10}$ units, and *L. monocytogenes* by 4. This was in contrast to the initial increase in numbers of *E. coli* O157:H7 and *L. monocytogenes*, 1-2 $\log_{10}$ CFU/g, which occurred for 2 days in fresh chicken manure held at 20°C. *S.e. Typhimurium* numbers remained stable during this period. Prolongation of the storage time to 6 days resulted in a 1-2 $\log_{10}$ decrease in *S.e.Typhimurium* compared to the initial count and a 3-4 $\log_{10}$ decrease in *E. coli* O157:H7; *L. monocytogenes* did not decrease below the initial counts. In the field after application of manure to pasture, Jones (1975, 1980) reported that survival times ranged from 2-3 weeks for *Salmonella*e on forage, but could be as long as 20-28 weeks in soil and fecal paddies.

Composting

Several types of composting can be used to treat manure (Rynk 1992). Often when manure is deep-stacked operators observe the generation of steam and heat from the stack and refer to this as composting. This undoubtedly is evidence of biological activity and heating, but it does not meet the requirements necessary for thorough disinfection. For composting to be used as a disinfection process, it needs to be conducted in a management context that includes specific time and temperature monitoring and mixing so that all parts of the piles are adequately heated. The method used depends on availability of equipment, land, type and amounts of manure, as well as supplements available for mixing into the pile (Rynk 1992). Types of composting include: bin, static pile, static force-aerated pile, windrow, in-vessel, tunnel, and channel. Time and temperature criteria applicable to biosolids disinfection by composting, i.e. specified in 40 CFR Part 503 (USEPA 1993), have become a benchmark for large-scale, nonbiosolids composting as well in several states and jurisdictions in the U.S.

Plym-Forshell and Ekesbo (1993) reported that *S.e.sv. dublin, S.e. sv. senftenberg* and *S.e. Typhimurium* survived less than seven days in composted cattle manure. In cold cattle manure, *S.e. sv. dublin* survived 183 days but not 190, while *S.e. sv. senftenberg* and *S.e. Typhimurium* survived 204 but not 214 days. In composted cow manure, *S.e. sv. senftenberg* and *S.e. Typhimurium* survived less than seven days while *S.e. sv. derby* survived 14 days but not 21 days. Lung et al. (2001) showed that *E. coli* O157:H7 and *S. enteritidis* were destroyed in cow manure composted at 45°C for 72 and 48 hr, respectively, in a lab-scale composter.

Multicomponent Treatment Systems

In addition to individual treatment processes, several groups of investigators have evaluated treatment systems that involve multiple steps of several treatment processes in tandem. Examples of these multicomponent systems are: 1) constructed wetlands (Duggan et al. 2001; Hill and Sobsey 2001); 2) manure solids separations-digestion-composting (Karpiscak et al. 2001; Vanotti et al. (2001; see also the section herein on Bacteria—Animal Waste Workgroup: Summary, Conclusions and Recommendations); 3) alkaline treatment combined with heat (Turner et al. 1999; Hogan et al. 1999; Millner, unpublished data); 4) acid-alkaline treatment of solids followed by composting (Millner, data not shown); and 4) thermophilic anaerobic digestion or bioreactor landfill digestion (Juteau et al. 2000; ten Brummeler 2000). As CAFOs implement more intensive levels of manure management technologies, the multicomponent systems may be expected to feature more prominently because they can provide solutions not only to nutrient management, but also to pathogen disinfection, air quality (odor and ammonia emissions), and product quality.

Avoiding Cross-Contamination

Regardless of the treatment method or system used for animal manure handling, it is fundamentally important for animal producers and manure managers to implement good management practices with regard to cross contamination between untreated and treated manure and animals infected with pathogens (Wells 2000). For example, farm equipment, such as tractors, that come into contact with untreated or partially treated manure and are then used in produce fields can be a source of contamination. Equipment used to turn compost, and other multiple use equipment that contacts manure, should be cleaned (such as with high pressure water or steam sprays) before it contacts fresh produce. Growers should also be aware

of other factors, such as farm layout and traffic flow, that may allow a tractor to drive through manure before entering a produce field. On-farm vectors by implements, animals, and human activities provide multiple opportunities for dissemination of manure pathogens beyond the sites of manure deposition or collection (Strauch and Ballerini 1994).

Summary

The main bacterial emerging and re-emerging pathogens of concern in manure are those that have been implicated in food- or waterborne illness outbreaks. Although a number of these organisms are clearly well-known fecal inhabitants, several also are ubiquitously distributed, and possibly traceable to specific wild animal sources in addition to livestock and poultry. Molecular methods that involve strain typing and detection are especially needed to address source and antibiotic resistance tracking.

The need to compare survival results from various treatment processes, farm practices, regions, and locations must be balanced against the inherent limitations of the currently available results. In reality, there is little data that is suitable for direct comparison because there is a persistent absence of standard sampling, sample handling, preparation and specific pathogen methodology that has been applied to manures, manured soils, and runoff. Methods that have been used appear to be appropriate for the individual studies and the reported results are credible. Furthermore, the sensitivity and robustness of methods are continually being improved. However, there is a pervasive lack of validation of the methods used for the types of complex matrices being examined. When considering the efficacy of treatments, the lack of results available for direct comparisons impairs the confidence of conclusions, particularly when studies show divergent results. Presently, results are useful in terms of relative comparisons.

As the emphasis and value of rapid, molecular methods grow for detection, isolation, and enumeration of bacterial pathogens, it will be necessary to validate the use and limits of the methods, so that results from different locations and laboratories can be confidently compared. The use of molecular methods for development of suitable source tracking procedures is an especially desirable goal. Source tracking would help determine the need to treat animal manure in specific situations. It would also help to determine the effectiveness of CAFO manure treatments relative to the wildlife sources that may contaminate or constitute a significant background at different seasons or locations. A broad range of molecular probes for these bacterial pathogens have been described in clinical and food microbiology peer-review literature. The task ahead will be to evaluate, adapt, standardize, and validate their use with manure, soil, and runoff water samples and to make the assays quantitative so that they are useful in evaluating the effectiveness of various treatment technologies. A number of treatment technologies individually and in tandem are capable of achieving a high degree of pathogen destruction in manure. The challenge will be to develop rapid, cost-effective, robust pathogen detection/enumeration systems that can meet the needs of microbiologists, the animal industry, and the environmental community.

With regard to antibiotic resistance, Teuber's review of the topic (1999) provides considerable evidence that the microflora of farm animals and food from them has acquired antibiotic resistance traits. Molecular genetic studies of the resistance genes, where available, demonstrate that humans and their food microflora are inseparable, and that conjugative transfer of resistance genes has occurred not only in vitro but also, in a few cases, in vivo. Haack et al. (2000) showed that mobile genetic elements, e.g., Tn916-like elements, occur in fecal enterococci from swine manure. Furthermore, these Tn916-like elements can transfer in the soil to a common soil microbe (e.g., *B. subtilis*), and because these same enterococci typically can survive and persist in field conditions for many weeks, there is sufficient time for transposable mobilization of antibiotic resistance genes exchange to occur. Thus, the potential for mobilization of additional resistance genes exists. Haack et al. (2000) only presented data for the transfer of tetracycline resistance, but it does raise questions about how many other resistance phenotypes are transferred, their rate of transfer, survival, and propagation through the conjugation events in the soil environment.

In conclusion, the increasing challenge of direct or virtual proximity pressure (via soil, water, air, or vectors) between animal and fresh fruit/vegetable production will continue to require diligence on the part of all producers to ensure that operational buffers and practices designed to resist microbial contamination are implemented and remain effective. In addition, the continuing emergence and re-emergence of pathogens will require vigilance and rapid identification/detection methods, and communication of results (Morens et al., 2004).

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Bacteria–Animal Waste Workgroup: Discussion Summary, Conclusions and Recommendations

Introduction

This workgroup focused on bacterial pathogens in animal manures. They decided to consider both human and animal pathogens in their assessment of priority pathogens and the research needed. The workgroup was able to identify and categorize specific pathogens as high, medium, or low priority.

Participants in this workgroup were: Jeffrey Karns, Leader; Pat Millner, Facilitator; Farid Bal'a; Elaine Berry; Al Dufour; J. Eric Line; Eliot Epstein; Richard Gast; John Haines; Gene Rice; Pat Scarpino; Perry Schafer; Thad Stanton.

Organisms

The workgroup began by generating a list of bacteria highlighted in keynote presentations earlier in the workshop. The list of organisms included: *E. coli* (O157 and other strains); *Salmonella; Listeria monocytogenes; Campylobacter; Helicobacter; Yersinia enterocolitica; Mycobacterium avium subsp. Paratuberculosis; Clostridium perfringens.*

Next, the workgroup discussed criteria for prioritizing these organisms. The criteria which they selected included incidence of disease, severity of illness or deaths attributed to the pathogen, association with food- or waterborne outbreaks due to manure contamination, occurrence in manure, the pathogen's persistence or survivability in the environment, the presence of significant sequellae, susceptibility to various treatments, source site ecology, and public perception (awareness and level of fear). The workgroup focused on one criterion at a time, and each workgroup member was given an opportunity to score each organism with respect to the criterion. The workgroup scored the organisms with a 'low,' 'medium,' or 'high' significance rating (rather than using a numerical ranking which may be skewed). The workgroup developed a matrix of organisms and criteria to record their scores. After deliberation, the selected organisms were given an overall priority ranking of high, medium, or low depending on the scores the organisms received on all the criteria. This particular workgroup did not have any epidemiologists involved in its discussion, so this ranking was considered a reasonable 'best effort' of the group based on the professional knowledge and materials available at the workshop. Workgroup participants noted that these rankings might change if additional epidemiological data became available, or if other published studies that they were not aware of were presented.

The workgroup considered both animal and human pathogens, so they also briefly addressed the animal diseases mastitis (caused by *Staphylococcus* spp.) and brucellosis (caused by *Brucella abortus)*. Participants discussed these two diseases not because they are significant public health issues, but because they thought it worth mentioning that framing microbial farm hygiene practices in terms of benefits to producers rather than as a government regulation would be more likely to gain acceptance. The workgroup also recognized that antibiotic resistant bacteria in manures and the impact of airborne endotoxin were two important additional issues that need to be considered in future research studies.

The consensus-based prioritization of bacterial pathogens by the workgroup is shown in Table 1 and constitutes their conclusions regarding which bacteria are of major concern.

Selecting Indicator Organisms

The workgroup considered a range of characteristics desirable in an indicator organism that would be used for monitoring pathogen reduction in a manure treatment process. However, members acknowledged that the actual organisms of concern may have to be used for survival and transport studies, because

attributes specific to each organism may be intricately involved in its movement and interfacing with environmental factors in soil, water, and air. In terms of indicator organism's attributes, first, the organism should be representative of bacteria found in manures. Next, the indicator organism should be chosen conservatively such that it is more resistant than the pathogens of concern to treatment, yet it must be susceptible to the primary modes of bacterial destruction. There should be reliable methods of detection for the organism, and it should be easy to detect.

Table 1. Ranking of Bacterial Pathogens of Concern

Priority Level	Organisms
High	Enterohemorrhagic *E. coli (EHEC); Salmonella spp; Listeria monocytogenes; Campylobacter*
Medium	*Yersinia enterocolitica; Clostridium perfringens;* other pathogenic *E. coli*
Low	*Mycobacterium avium* subsp. *paratuberculosis*

The workgroup discussed the use of different organisms like *E. coli, Clostridium, Salmonella,* and *Enterococcus* as indicators of treatment process effectiveness. Certain organisms, particularly fecal coliforms and *E. coli,* were not considered the best choices because they may be too sensitive or not always present (e.g., *E. coli* may be a bad indicator for use with poultry manure because the 0157 strain of *E. coli* has not been found in chickens). There was also a suggestion that using *Clostridium* as an indicator may be setting the bar too high (as it may be too hard to inactivate because of its ability to produce thermostable spores). Another issue discussed was whether or not there should be a different indicator for different animal manures or if one could cover all manures. The workgroup eventually selected *Enterococcus* as an indicator because it met most of the criteria set by the workgroup:

- Representative of pathogens, but conservative (as or more resistant/persistent than pathogens);
- Universally found in manures;
- Easy to detect with a simple and reliable method, and susceptible to treatments using chemicals and/or heat.

Workgroup members suggested that treatment process criteria analogous to those described in 40 CFR Part 503 for biosolids treatment processes might be suitable models to consider. However, it would be necessary in validation studies to carefully define the relationship between the indicator and the various pathogens throughout an entire process.

The workgroup participants concluded that fecal coliforms or *E. coli* may not be satisfactory as treatment process effectiveness indicators because they are not always present in manures and because they might be more sensitive to inactivation than are bacterial pathogens. They further concluded that *Enterococcus* may be a satisfactory indicator of treatment process effectiveness. For studying the survival and transport of bacterial pathogens in manures, it may be necessary to use the pathogen itself rather than an indicator.

Ability To Assess Risks

Workgroup participants concluded that there was insufficient information to conduct a risk assessment at this time. They also concluded that they could not assess the risk (or effectiveness) of the various treatment processes accurately.

Detection/Analytical Capabilities

The workgroup addressed several issues regarding sampling of individual pathogens. One issue was the size of samples needed in order to effectively detect organisms. They were concerned that in some situations pathogens may be present, but in small amounts that would require collection of large samples for analysis. The question of sample size is tied to desirable detection limits. Even with polymerase chain reaction (PCR) methods, detection still requires greater than 10^3 organisms/gram.

With enrichment, it is expected that 10 to 1000-fold better detection would be possible than with direct detection without enrichment. However, this involves a trade-off in that directly quantitating from the immediate sample is lost. With molecular techniques presently available, direct detection (without pre-enrichment) is as sensitive quantitatively as the culture method, but molecular techniques allow strain-specific identification and more rapid results than the cultural techniques. Improved molecular techniques may eventually exceed the sensitivity of cultural techniques.

Participants discussed using quantitative versus qualitative (presence/absence) reporting of pathogens, and concluded that reporting should be specific to the question being asked and the limit being applied. Quantitative data using uniform sampling, sample handling/processing, and analytical protocols are needed to validate treatment and support risk assessment. There are no such data currently for any of the priority bacteria listed in Table 1. Another difficulty is that not all pathogens are easily and rapidly culturable (e.g., *M. avium* subsp. *paratuberculosis).* The availability of molecular and immunological techniques that may complement or replace culturing for the bacterial pathogens that were considered is shown in Table 2.

Table 2. Existence of Techniques for Detection/Analysis of Bacterial Pathogens

Pathogen	Molecular Techniques	Immunological Techniques
Campylobacter	Yes	Yes
E. coli	Yes	Yes
Listeria	Yes	Yes
Mycobacteria	Yes	Pending
Salmonella	Yes	Yes
Yersinia	Unknown	Unknown

Detection/Analytical Capabilities Conclusions and Recommendations

The following conclusions and recommendations were made by the workgroup regarding detection/analytical capability issues:

- *Sample size:* Should be based on the desired detection limit; Extraction techniques are currently limiting; and Work needs to be done on isolation techniques.
- *Molecular/immunological techniques:* Exist or can be developed to detect the pathogenic forms of most of the bacteria of concern; These techniques may complement or replace cultural techniques; and Cultural techniques demonstrate that the organism is capable of reproduction.
- *PCR Techniques*: Detection limit still > 10^3 organisms/g; Extraction techniques are limiting; Immunomagnetic bead separation (IMS) is currently a costly, but promising technique if antibody is available.
- *Reporting:* Should be standardized to dry weight basis.
- *Strain Diversity:* Research is needed to understand diversity in strains and modeling data.

Treatment, Process, and Control Technologies

The workgroup listed known treatments currently available (Table 3) and, in some cases, in use (such as lagoons, liming, and composting). The technologies were characterized with a high/low rating based on factors such as general performance, effectiveness in pathogen reduction capacity, and overall capital and operating costs. If the effectiveness of a treatment was not known, this was identified as a data gap. Only on-site treatments at farms and concentrated animal feeding operations (CAFOs) were evaluated; no attempt was made to consider regionalized facilities or any accompanying transportation issues. However, the costs relative to the size of the animal production operation were considered. The expense for trans-

Table 3. Characterization of available manure treatment technologies with regard to relative reduction in fecal coliforms, lethal stressors, solids handling capacity, cost, vector issues, odor, endotoxin, and worker exposuure

Treatment	Log[a]	Stressor[b]	Solids[c]	Cost	Vectors[d]	Odor[e]	Endotoxin Issues Process/ Product[f]	Worker[g]
Lime treatment	2-5	pH; heat; ammonia	H	M	L	M-H	L / ?	L
Lagooning	1-4	time; temperature; bioactivity nutrient loss	L	L- M	H: birds, insects, mosquitoes, rodents	H	L / ?	L
Stacks	?	ammonia, heat	H	L	H: birds, flies, beetles, rodents	L-M	H / ?	M-H
Air drying	1-2	dessication	L-H	L	H: flies and other insects	L-M	H / H	L
Composting	1-5	time-temperature; ammonia; nutrient loss	H	L- M	L - M	L-H	H	H
Digestion - Anaerobic Mesophilic Thermophilic	 2 5	time; nutrient lost + bioactivity; heat + bioactivity	 L L	LH	L: enclosed process	 L L	L / ?	L
Digestion -Aerobic Mesophilic Thermophilic	 1-2 5	time; nutrient lost + bioactivity, heat + bioactivity	 L L	 L H	 H L	M	M / ?	M - H
Pasteurization	5	time; heat	L	H	L- M	L-M	L / H	L
Heat drying	4-5	temperature; dessication	H	H	L	H	VL / H	M
Constructed Wetlands	2-5	bioactivity	L	M	L: subterranean; H: surface	M	L / L	L

[a] Log_{10} reduction of fecal coliform populations; consensus agreement of typically good performance for each treatment process; [b] stress mechanism most responsible for bacterial destruction; [c] L= low, M = medium, H = high, VL=very low: relative ratings for 1) suitability of the process in handling solids content, 2) costs, 3) potential vector attraction and transmission to off-site locations, 4) potential for malodors during treatment, 5) generation of airborne endotoxins during a treatment process and during use of the product, 6) potential for worker exposure to bacteria and endotoxins during treatment

? = unknown

porting manure off-site may be costly for large operations. Small operations with crops can also contribute to pathogen problems, so the challenge to the scientific community is to come up with economically viable solutions and benefits for them. These solutions would conceivably include more biologically-based approaches that incorporate limited amounts of external inputs, utilize and optimize the output quality, provide cost effective advice for the producer, and increase public education about what treatment accomplishes. Table 3 summarizes the discussion and conclusions about existing treatment technologies and their impact on bacterial survival in manure.

The workgroup discussed regrowth of pathogens. It was suggested that processes from biological activity should have less problems with regrowth. It was also suggested that those processes that leave carbon behind can have a significant effect on regrowth if the treated material gets wet or suffers mishandling. Microbiological deterioration of product quality is also often noticeable by the production of malodorous compounds.

With respect to antibiotic resistance, it was generally acknowledged that if sufficient pathogen destruction is accomplished during processing, chances for introduction of antibiotic resistance are lessened.

In addition to currently available technologies, two members (Vanotti and Millner) of the workshop noted that a number of technologies were known to be under current evaluation at several centers in the U.S. and Asia. They provided preliminary background information and data on a multistage treatment process that they have been involved with in North and South Carolina for swine manure. This process involves a solids separation step with polymer addition, followed by nitrification and denitrification steps, and a final phosphorus precipitation step using lime. Results of liquid phase analyses at each step show significant reduction in total and fecal coliforms and enterococci with solids separation (67 to 83%) accompanied by 98% removal of volatile suspended solids. Biological nitrogen removal with alternating anoxic and noxic conditions resulted in reductions of 5 $\log_{10}$ (99.997%) of total coliforms, 4 $\log_{10}$ of fecal coliforms and enterococci, and 2 $\log_{10}$ of salmonellae.The final lime treatment to precipitate phosphorus achieved a pH 10.3 and eliminated detectable salmonellae to below threshold levels of 30 colony-forming units/mL of effluent. Thus, the multistage treatment process shows significant promise in elimination of pathogens in effluents from swine manure treatment. Solids were composted and with good process control showed equally good effectiveness in reduction of pathogens in the final horticulturally suitable product.

Conclusions

The participants did not attempt to provide an overall ranking of the 10 treatment process approaches summarized in Table 3 because suitability of a process technology is dependent on a multitude of factors and site-specific considerations. Table 3 summarizes the relative effects on fecal coliform reduction, endotoxin issues, odor, vectors, and costs for each technology considered.

The workgroup noted that pathogen regrowth issues will be process related. Those processes that leave carbon behind (alkaline stabilization and drying) can have a significant effect on regrowth if the product gets wet or is inadequately handled during storage and land application.

Products from biological activity processes should have fewer problems with regrowth.

Antibiotic resistance—If sufficient pathogen reduction is accomplished during processing, chances for introduction of antibiotic resistance determinants are lessened, but the extent of their survival and transmission has yet to be examined and quantified.

Pathogen reduction efficiencies for different treatment technologies have been reported by a wide range of laboratories over a considerable period of time (in the US and internationally). However, no standard or uniform sampling, sample processing and handling, or set of analytical protocols has been used. Therefore, precise efficiencies and log reductions for fecal coliforms or other bacteria of concern, e.g., *Salmonella,* are presently unavailable; values in Table 3 should be considered as general estimates.

Prioritized List of Research Needs

The workgroup agreed on the research needs listed below relative to bacterial pathogens in animal manure. These needs were viewed as equivalent, so some effort should be devoted to all of them concurrently. However, the workgroup acknowledged that fundamental to all of these, there was a need to develop and use at least some standard culture methods, possibly by a core group of investigators involved in the treatment, fate, and transport studies. Research needs:

- The reduction potential of the treatment processes for the different pathogenic bacteria is largely unknown. Therefore, determining time, temperature, and concentration relationships for each pathogen for the treatment processes is necessary. A question was raised as to whether measurements of temperature or ammonia concentration could serve as process monitors once the reduction potential of such processes was determined.
- Determining the relationship between pathogen behavior and that of the indicator species in treatment processes.
- Developing robust molecular, immunological, IMS/culture techniques for detection of pathogenic bacteria at acceptable limits of detection

SECTION III

Viruses in Biosolids/Treated Sewage Sludge and Animal Waste

Enteric Viruses in Biosolids

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Introduction

In 1993, the United States Environmental Protection Agency promulgated the 503 regulations which established rules for the disposal of sewage sludge or biosolids (USEPA 1993). In this rule, Class A and Class B biosolids were defined. (*Editor's Note: See paper by J.B. Farrell, these proceedings, for a detailed discussion of Class A and Class B requirements under the 1993 40 CFR Part 503 regulations.)* Class B biosolids must be treated by "processes which significantly reduce pathogens" and Class A by "processes to further reduce pathogens." All land applied biosolids must meet at least Class B requirements. Class A biosolids requires that the enteric virus concentration be less than one plaque forming unit per 4 grams total dried solids. Thus, enteric viruses were recognized in these regulations as a potential contaminant whose concentration must be controlled to provide safe utilization. Since the development of these regulations, several new enteric viruses have been recognized and better methods have become available for the growth and detection of enteric viruses. In addition, the development of quantitative microbial risk assessment in the last 10 years has allowed us to better understand the risks posed by viruses and to sensitive populations (Haas et al. 1999). Unfortunately, little research has been conducted on the occurrence and fate of enteric pathogens in biosolids in the last decade, leaving many health risk questions regarding viruses unanswered.

Enteric Viruses in Biosolids

There are more than 140 different types of enteric viruses excreted by man that can find their way into domestic sewage and biosolids (Table 1). Of these, the environmental fate of enteroviruses has been studied the most because they are easily grown and assayed in the laboratory. Other viruses, such as the caliciviruses (norovirus and saprovirus), we know little about since they have not yet been grown in the laboratory. Enteric viruses affect almost all organs of the body. The enteroviruses are the largest group of enteric viruses causing some of the more serious illnesses, particularly in children. Overall, children are more likely to suffer serious illness, greater attack rates, and mortality from enteric infections than any other population (USEPA 2000).

Enteric viruses are the major cause of childhood diarrhea in the United States, the leading cause of childhood hospitalization for gastroenteritis when an agent can be identified, and they result in an estimated 100 deaths per year related to gastroenteritis. Rotaviruses and caliciviruses are the leading causes of gastroenteritis in the United States (Monroe et al. 2000). Rotaviruses and caliciviruses have been shown to be transmitted by food and water. Caliciviruses may be the most significant cause of water and foodborne illness in the world (Monroe et al. 2000). Enteric adenoviruses are the second most common cause of childhood viral diarrhea. While causing a mild diarrhea in children, mortality of immunocompromised persons (e.g., those receiving immunosuppressive agents due to organ transplantation, cancer chemotherapy patients, AIDS patients, etc.) range from 53 to 69% (Gerba et al. 1996). Other adenoviruses cause nose, eye, and respiratory infections. Astroviruses are also a cause of gastroenteritis and have been shown epidemiologically to be transmitted by food and water (USEPA 2000).

Non-polio enteroviruses are estimated to cause 10 to 15 million symptomatic infections in the United States annually (Zaoutis and Klein 1998), with estimated case fatalities of 0.001% to 0.94% (Assaad and Borecka 1977). They are associated with a broad spectrum of clinical syndromes, including aseptic meningitis, herpangina, hand-foot-mouth disease (vesicular eruptions of the palms, soles and mouth caused by coxsackieviruses and enterovirus 71–different from Foot and Mouth Disease in animals which is caused

Table 1. Human Enteric Viruses

Virus Group	No. Of Types
Enteroviruses Poliovirus Coxsackievirus Echoviruses Enteroviruses (types 68-71)	69
Hepatitis A virus	1
Reoviruses	3
Rotaviruses	8
Adenoviruses	49
Astroviruses	7
Hepatitis E virus	1
Caliciviruses (norovirus; saporovirus)	2

by an aphthovirus), conjunctivitis, pleurodynia, myocarditis, poliomyelitis, rashes, neurological disorders, and diabetes. While all age groups are affected, the most serious outcomes are in the newborns, young children, and young adults.

Hepatitis A and E primarily infect the liver. Each year, approximately 140,000 persons in the U.S. are infected with hepatitis A virus, with an annual cost of between $332 million to $580 million (Berge et al. 2000). The highest rate is among persons 5 to 14 years old (CDC 1999). Hepatitis E virus is the leading cause of acute viral hepatitis in developing countries. In contrast to hepatitis A, hepatitis E largely causes clinical infections in young and middle age adults. Hepatitis E is a more serious infection than hepatitis A with case fatalities of 2 to 3% in the general population and 20 to 30% in pregnant individuals (Haas et al. 1999).

Emerging enteric viruses, which have been recently recognized, are the picobirnaviruses, picotrirnaviruses, coronaviruses, and toroviruses. Human picobirnaviruses and picotrirnaviruses were originally detected in the stools of AIDS patients. Picobirnaviruses are a novel group of viruses which cause diarrhea in AIDS patients, travelers, children, and the elderly (Bouza et al. 2001). They belong to the family Birnaviridae. These viruses are similar in size to the caliciviruses but have bisegmented and trisegmented genomes. They cause infections in both man and animals (Chandra 1997). Enteric coronaviruses have most frequently been associated with gastrointestinal disease in neonates and children younger than 12 years old. Toroviruses are most commonly associated with gastroenteritis in immunocompromised persons and older children (Jamieson et al. 1998).

In summary, over the past decade the types of illnesses attributed to enteric viruses have increased, as well as the number of agents. Recent research has suggested that caliciviruses may be the number one cause of food and water borne outbreaks in the developed world.

Occurrence of Enteric Viruses in Biosolids

Unfortunately little research has been done on the occurrence of enteric viruses in biosolids for almost a decade. The existing database is almost exclusively for the enteroviruses. Straub et al. (1993b) reviewed the existing information up to that time. The literature suggested that the concentration of enteric viruses (enteroviruses) ranged from 10^2 to 10^4 per gram dry weight of solids in raw sludge and an average of 300 per gram in secondary treated sludge. The secondary treated sludges had been treated by processes that would be considered Class B under the 503 regulations. Aerobic and anaerobic digestion are the processes most commonly used to produce secondary biosolids. The inactivation of enteric viruses by treatment processes is usually pH and temperature dependent. In general, a plant using mesophilic digestion (37° C) with a mean retention time of 10 to 20 days can reduce enterovirus concentration by 90% (one $\log_{10}$). Anaerobic digestion can result in a similar reduction (Table 2). Our experience in monitoring Class B biosolids since the 503 regulations have been in effect is that the concentration of enteroviruses in Class B is now significantly

less probably because of better management of the treatment process. We generally find that Class B biosolids produced by anaerobic digestion contain less than one virus per four grams. The secondary sludges had been treated by processes that would be considered Class B under the 503 regulations.

The current methodology used for the detection of enteric viruses and assay procedures has largely been optimized for the enteroviruses (Goyal et al. 1984). Methods are needed that have been shown to be efficient for the wide range of other enteric viruses, which may be present in biosolids.

It should be emphasized that for many processes the amount of data on removal by various processes is very limited. Data our laboratory has collected over the last ten years on anaerobic mesophilic digestion would suggest that on average a 90% removal or more of enteroviruses is achievable by these processes on a routine basis. Data on other viral groups is almost non-existent. The presence of enteric adenoviruses (Gerba unpublished) and astroviruses (Chapron et al. 2000) has been recently demonstrated, but little is known about occurrence and removal by various sludge treatment processes.

Table 2. Summary of Enterovirus Reduction during Sludge Treatment

Treatment	Reduction (log_{10})
Anaerobic digestion (mesophilic)	1
Aerobic	1
Composting	2-3
Air drying	1-3
Lime Stabilization	3

Fate of Enteric Viruses in Land Applied Biosolids

In 1999, the EPA (USEPA 1999) estimated that 41% of the 6.9 million dry tons of biosolids generated nationwide in 1998 was land applied. This is expected to increase to almost 50% by the year 2010. In Arizona, over 95% of the generated biosolids are land applied to agricultural lands (Horowitz 2001). However, opposition from citizens, environmental groups, and farmers adjacent to lands to which biosolids have been applied is growing nationwide. The concern has largely been from the lack of information on the presence and fate of environmental contaminants. Little data is available on the survival of enteric viruses in biosolids. Temperature and moisture are the primary factors governing the survival of enteric viruses in soil. Sorber and Moore (1987) were able to recover enteric viruses from a biosolid burial site in Montana six months after the last application. In Denmark in the winter, polio and echoviruses were found to survive up to six months in biosolids applied to sandy soils (Damgaard-Larsen et al. 1977). In Florida during the dry season, Bitton et al. (1984) showed that enteric viruses were inactivated eight days after biosolid injection into the soil and suggested that a combination of evaporation and warm (27°C) soil temperatures would significantly reduce risks. Straub et al. (1993a) studied the survival of the coliphages MS-2 and PRD-1, and poliovirus type 1 in two agricultural soils from southern Arizona at various temperatures ranging from 15 to 40°C. Clay soils afforded more protection to the three viruses than sandy soil. Evaporation to less than 5% soil moisture completely inactivated all three viruses within seven days at 15°C and 2 days at 40°C, regardless of soil type. This suggested that a combination of high soil temperature and rapid loss of soil moisture can significantly reduce risks caused by viruses in biosolids amended soils.

Gerba et al (2002) performed a risk analysis to asses the risk of enteric virus infection from accidental exposure to Class B biosolids after land application. Data on the occurrence of enteric viruses over a four year period from a local anaerobically digested Class B biosolids product was used. To simulate worst case conditions, only samples in which enteric viruses were present were used in the analysis. The samples in which viruses were detected contained an arithmetic average of 5.18 viruses per four grams dry weight. After incorporation of the biosolids into the soil as required, the risk of virus infection to a child after playing in the soil for eight hours, was estimated to be from 10^{-5} to 10^{-7}. These risks are below the 10^{-4} for infection by drinking recommended by the United States Environmental Protection Agency. While human access is actually restricted for some time after land application of Class B biosolids, this type of analysis suggests that risks from even non-intentional exposure to biosolids for a prolonged period of time are low.

Of all the enteric pathogens in biosolids, viruses have the greatest potential for contamination of groundwater. Enteric viruses are the major cause of groundwater disease outbreaks in the United States when an agent can be identified (Abbaszadegan et al. 1999) and have been detected in almost one-third of the drinking water supply wells in the United States (Abbaszadegan et al. 1999). Studies with enteroviruses would suggest that they are tightly bound to biosolids and migration through the soil is limited (Damgaard-Larsen et al. 1977; Bitton et al. 1984). These studies used cell culture infectivity assays and the results would suggest that virus migration from biosolids applied to land is limited to a few centimeters. This is in contrast to the land application of domestic wastewater where vertical transport of greater than 30 m and horizontal transport of 150 m have been documented (Bitton 1999). Using the polymerase chain reaction (PCR), Straub et al. (1995) were able to detect enteroviruses in soil samples up to two meters (the maximum depth investigated) below the soil surface where Class B biosolids had been applied. Soil samples from lower depths were not collected. The authors suggested that the combination of spring rainfall and irrigation might have contributed to the migration of the viruses into the soil. It was also pointed out that PCR does not indicate that the detected viruses were infectious. None of the PCR-positive soil samples were positive by cell culture infectivity assays. Still, the results suggest that virus migration to two meters or greater is possible from land applied biosolids. Biosolids had been applied to this farmland for over ten years. No samples of groundwater in this area yielded infectious virus; depth to groundwater was approximately 100 ft (unpublished data).

The transport of viruses through the soil is controlled by the physical-chemical characteristics of the specific virus, climate, and the composition of the soil. The size of the virus, its isolelectric point, inactivation rate, nature of the soil, temperature, soil moisture, soil and water pH, soil mineral composition, rainfall events, and soil pore size play a role in how far a virus will migrate. Long distant transport of viruses in sandy soils is possible under saturated conditions (10 meters or more). Until recently, transport through unsaturated soils was thought to be limited, however, significant transport through unsaturated soil was recently reported by Jin et al. (2000) and Chu et al. (2001). Of particular concern was that some viruses may be more readily inactivated at the soil-water interface than others. For example, coliphage MS-2 was found to be held up more at the air-water interface under unsaturated conditions and inactivated to a greater degree than coliphage phiX-174.

Microbial aerosols are generated during the land application of biosolids. The degree of aerosolization is dependent upon the method of application (spraying, chain dragging, and manure spreader); the percent suspended solids; and environmental conditions (wind speed, relative humidity, sunlight). Enteric viruses have been detected during the spray irrigation of unchlorinated domestic wastewater and enteric bacteria in aerosols generated by activated sewage treatment plants (Bitton 1999). Sorber et al. (1984) studied microbial aerosols generated during the land application of anaerobically digested biosolids. Of the four sites studied, two used tank trucks and two used spray guns. While enteroviruses were detected in the biosolids, none were detected in the air samples. However, increased levels of bacteriophages, coliforms, fecal coliforms, and fecal streptococci were detected 50 meters downwind. A pooled sample volume of 1470 m^3 of air was tested for enteric viruses. This suggested that the viruses were present at a concentration of less than 0.0016/m^3. The authors concluded that aerosol concentrations at biosolids application sites are less than those at wastewater spray application sites, and should have no adverse health effects. Using all glass impingers to collect aerosolized microorganisms (Dowd et al. 1997), only detected coliforms on one occasion and no fecal coliforms at a site where biosolids were applied by a manure spreader (Pillai et al. 1996). However, thermotolerant clostridia and bacteriophages were detected in 73% and 53% of the samples, respectively. At this site the dried biosolids were spread on the land by tossing them 50 meters through the air (Pillai, personal communication). Using ribotyping, the source of the thermotolerant clostridia was determined to be the biosolids (Dowd and Pillai 1999). Using the data on the concentration of bacteriophages in the air with aerosol and risk models, Dowd et al. (2000) estimated the risk of infection to persons at different distances from the site of application. Under low wind velocities (2 meters/second) and a one-hour exposure, the risk of viral infection to workers at the site was determined to be 3:100 at distances up to 500 meters. Risks at distances of greater than 500 meters were not considered significant under normal wind conditions. It should be recognized that the risk estimates in that study were worst case in terms of concentrations of the pathogens in the biosolids, application practices, and atmospheric conditions. Recent studies by our group have found that aerosolization of viruses is significantly less in biosolids compared to water during application via spray. The binding of the viruses to the biosolids may be acting to prevent their aerosolization. Application of biosolids by manure spreaders and slingers produce even less aerosolization because of the high solids concentration.

Summary and Recommendations

We actually know surprisingly little about the occurrence of enteric viruses and their removal by biosolids treatment processes. Most of our knowledge comes from work done before 1990 and is generally limited to the enteroviruses. Enteroviruses may represent a small fraction of all the viruses present in biosolids. Previous cell culture techniques were very limited in their ability to detect viruses in wastewater. Based on evidence from cell culture assay efficiency, immunoassays, and PCR, the actual number of enteric viruses in sludge is probably 100 to 1,000 times greater than was determined by previous methods (Blackmer et al. 2000; Ward et al. 1984). For proper risk assessment we need to get better estimates on concentration of enteric viruses in biosolids, otherwise all we are doing is guessing. Information on the resistance to inactivation of hepatitis A virus and adenoviruses is needed. These are the most thermally resistant viruses and survive longer than enteroviruses in the environment (Enriquez et al. 1995). Data on caliciviruses are also needed. They are now believed to be the major cause of food and water borne disease in the developed world (Monroe et al. 2000).

Surrogate indicators are needed to better assess biosolids treatment processes and fate of pathogens after land application. Such an approach will give better assurances of the adequacy of treatment and safety after land application.

Better assessments of exposure via aerosols and groundwater are needed. Through the use of indicators we may be better able to get an idea of exposure via aerosols generated during handling and land application. We need to assess the potential for viruses to be transported through soil after biosolids application, especially under unsaturated conditions. A national survey of viruses in Class A and B biosolids is needed so that quantitative microbial risk assessment can be used to define the risks of viruses in biosolids. Research needs are summarized below:

- Application of newer methods to determine occurrence of known and emerging viruses in biosolids
- Removal of known and emerging viruses by sludge treatment processes
- Development of microbial indicators of sludge treatment performance for the removal of viruses
- Development of surrogates or models to determine survival of viruses after land application
- Modeling of virus movement through soil after land application of biosolids
- Studies are needed on occurrence and treatability of biosolids for adenoviruses, hepatitis A virus, rotaviruses, and caliciviruses
- National survey on the occurrence of enteric viruses in Class A and B biosolids

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Viruses-Sludge/Biosolids Workgroup: Discussion Summary, Conclusions and Recommendations

Introduction

This workgroup identified a number of viral disease agents that should be considered in the management of biosolids. The group chose to generally exclude blood-borne viruses and those viruses that infect through the skin. In terms of indicators (virus surrogates), this workgroup suggested a tiered approach in which rapid tests with conservative screening indicators could be followed by more time-consuming and more specific indicators. In addressing the ability to assess risks, the workgroup considered occupational exposures as well as exposure of the general public. Issues related to Class A and Class B biosolids were explored. Sampling and analysis guidance, and matrix effects on virus recovery, were two issues examined while discussing detection and analytical capabilities. The relative resistance of viruses to stressors that affect treatability was discussed. A list of research needs on viruses in biosolids was developed.

Participants in this work group were: Charles Gerba, Leader; Robert Bastian, Facilitator; Don Brown; Mary Ann Bruns; Nancy Burton; Joe Eisenberg; Donna Francy; Alan Godfree; Cecil Lue-Hing; Aaron Margolin; and Nina Sweet.

Organisms

Table 1 lists the human viruses considered in assessing the risks associated with biosolids application. In discussion of the list, it was agreed that viruses that infect animals should also be included. The purpose of the list was to consider all possible viruses and then eliminate those of lesser significance. Biosolids treated by Class A and Class B processes were considered in determining what should be included in the list.

During derivation of this list, the workgroup made a few notable exclusions. The workgroup concluded that generally blood-borne viruses, e.g., HIV, were unlikely to be an issue in biosolids because they would be inactivated during wastewater and/or sludge treatment. The workgroup did not consider viruses that cause infection by the dermal (skin) route. Lipid-containing viruses have a low viability in aqueous environments and may not survive wastewater and/or sludge treatment. However, some lipid-containing viruses, e.g., rhinoviruses, influenza viruses, and herpes viruses, could be considered until it has been determined whether or not they survive treatment. While many of the viruses listed in Table 1 have not been reported in sewage or biosolids, they may potentially be present.

Indicator Organisms

The workgroup listed five issues that should be taken into account when selecting indicators (surrogates) for viruses: Treatment performance; Cost of analysis; Length of time needed to get results; Sensitivity of the test; and Specificity. Table 2 is a list of agents (organisms and physical/chemical parameters) that could serve as possible indicators of treatment performance and post treatment risk.

When developing indicators for viruses in biosolids, a tiered process for treatment performance may be considered. As mentioned above, the length of time needed for obtaining results is an issue with some of the indicators considered. For example, the assay for enteroviruses currently takes between 10 to 15 days while fecal coliform results may be obtained in less than 24 hours. If fecal coliforms are reduced by treatment, this indicator would assume that other organisms also should be reduced. It was suggested in the workgroup that perhaps there should be two groups of indicators: one group containing indicators that may be less accurate but quicker to detect and enumerate, and another group of indicators that are more accurate and inclusive but take longer to produce results. Under this approach, indicators would be prioritized into tiers with the first level being targeted towards conservative indicators that are capable of rapid analysis.

Table 1. Viruses Considered for Risk Assessment of Biosolids Application Programs

Virus Group	Have been isolated from sewage/biosolids	Comments
Enteroviruses	Yes	It has been determined through cell culture that they can exist and survive for long periods of time in the environment
Rotaviruses	Yes	Cell culture has been developed to determine viability
Adenoviruses	Yes	It has been shown by cell culture that they can withstand heat and survive for longer periods of time in the environment. Probably the longest surviving of all viruses likely to be present in biosolids. One waterborne outbreak reported in Finland
Hepatitis A virus (HAV), Hepatitis E virus (HEV)	Yes	Cell cultures have been developed to determine viability. HAV has been found to withstand moderate heat and survive for long periods of time in the environment
Caliciviruses	Yes	
Astroviruses	Yes	Cell culture has been developed to determine viability
Reoviruses	Yes	Cell culture has been developed to determine viability
Syncytial Respiratory Virus	No	
Aichivirus	No	First described in Japan following outbreak of non-bacterial gastroenteritis associated with consumption of oysters
Picobirnavirus	No	
Picotrirnavirus	No	
Toroviruses	No	Affects children and the elderly
Parvoviruses	No	Can cause skin infections
Coronaviruses	No	Said to possibly affect children, but no reliable prevalence data
Hepatitis B virus (HBV)	No	May not be present in sludge, however, can persist for approximately a week in the environment
Human immunodeficiency virus (HIV)	No	Blood-borne virus
Foot and Mouth Disease Virus (FMDV)	No	Animal virus
Rhinoviruses	No	Respiratory viruses

As the workgroup discussed treatment process indicators and verification of treatment, they considered that the list of possible indicators should be examined to see if any one particular agent could meet all needs (the one indicator approach is where the science is now) as opposed to using a multiple indicator approach. *E. coli* may be an appropriate single indicator and is gaining acceptance in Europe. However, the group considered that more work needed to be done to confirm the correlation between the behavior of *E. coli* and viruses in sewage and sludge treatment.

Other indicators issues included: Determining if current indicators are still valid; Determining relative risk or association with other enteric viruses; Investigating whether or not a bacteriophage indicator is rel-

Table 2. Suitability[a] of Select Agents as Indicators of Treatment Performance and Post Treatment Risk for Viruses

Agent	Treatment Process	Post Treatment Risk
Adenoviruses	?	?
Ascaris	+	+
Coliphages	+	+
Clostridium perfringens spores	+	+
Enterococci	+	-
Enteroviruses	+	+
Escherichia coli	+	-
Fecal Coliforms	+	-
Lime (pH or alkalinity)	+	+

[a] "+ " = the agent would be an acceptable indicator; "-" = the agent would be an unacceptable indicator; "?" = not certain whether the agent would be an acceptable indicator.

evant; and The use of physical/chemical indicators. Table 2 does not include all possible chemical indicators; lime (or pH) was the only physical/chemical indicator included here. The workgroup suggested that perhaps other chemical indicators should be considered (e.g., ammonia). The effectiveness of sludge treatment processes which rely on physical methods of treatment (e.g., pasteurization and thermal drying) can be monitored on-line in real time, obviating the need for routine microbiological analysis.

Ability To Assess Risks

The following risk assessment issues were considered by the workgroup: Risks to occupational groups and the general public; Whether or not Class A biosolids can be considered "virus-free" if they satisfy the current requirements of the 503 regulations; Bioaerosols and dust; Vectors; Depth to groundwater; Adequacy of public access/site restrictions, grazing access and signage requirements for Class B application sites; and The risk from a one-time accidental exposure.

The workgroup considered health risks to workers, including special groups of workers such as sewer cleaners who may be exposed to raw (untreated) wastewater. These workers are obviously potentially at a greater risk of contracting infections from the many viruses present in raw wastewater than the general public would be. The workgroup also considered the risk of exposure, especially for Class B biosolids, by inhalation where aerosols or dust are created in the treatment plant or by land application of biosolids in the field. Possible solutions for reducing occupational risk include: Increased education for workers; Imposing additional safety requirements (e.g., personal protective equipment, vaccinations); Imposing minimum requirements through additional site-specific guidance and permits; and Conducting risk analyses that consider particle size and moisture content.

The workgroup also addressed the need to assess the risk of virus transmission by vectors such as flies. While Class A biosolids should not produce any significant risks, flies attracted to Class B biosolids could pose a problem. Vector attraction reduction, for example, by means of digestion to achieve 38 percent reduction in volatile solids, may not prevent vector-borne transmission of viruses because treated biosolids, which may contain viruses, remain attractive to flies and other insects. Although there is a general relationship between viruses and vectors, and between flies and disease, it was suggested that there is insufficient information about the risk of disease transmission from flies visiting Class B biosolids.

Class A and B Biosolids Issues

The workgroup discussed concerns about the risk from land application of biosolids to sites used to produce food crops. There were questions raised about how confident we should be that Class A is vi-

rus-free. Since the definition of Class A is that viruses are below detectable limits, the risk may appear to be greater with the advent of new/improved detection methods. There was also concern expressed over the appropriateness of the current required waiting periods prior to the production of food crops on sites where Class B biosolids had been applied and the 2 million fecal coliform per gram level used to define Class B biosolids.

The workgroup also addressed concerns related to access of humans and animals to places where Class B biosolids have been applied. The 30-day waiting period for public entrance to lands where Class B biosolids has been applied was discussed; Some workgroup members had reservations about the protectiveness of this time restriction against exposure to viruses. Concerns were expressed about the need for measures to protect against unanticipated public entrance and exposure. Although warning signs and fencing requirements were offered as possible solutions, it was suggested that a risk assessment is required to address these situations to determine the risk of one-time accidental human exposure to Class B biosolids. Questions were also raised about what would constitute an acceptable risk for a one-time exposure event to Class B biosolids. Similarly, for animal grazing access, there was some uncertainty about the adequacy of a 30-day waiting period following the application of Class B biosolids.

The workgroup would like to see setbacks addressed, e.g., from a regulatory perspective, are the minimum requirements in the 503 regulations appropriate for all states, or should setbacks be controlled at the state level? It was suggested that perhaps permits should be issued to each site indicating the biosolids that could be used, the lands they could be applied to, and the restrictions necessary to meet appropriate risk levels that the scientific community helps to determine. Minimum requirements could be imposed, and through guidance, additional limits that might be appropriate through site-specific permitting could be suggested. Guidance could also be used to establish appropriate controls for application of Class B biosolids.

Detection/Analytical Capabilities

The workgroup expressed two main concerns with respect to detection/analytical capabilities: 1) The need for appropriate sampling and analysis guidance; and 2) The need for more information regarding matrix effects on methods for virus recovery.

Sampling and Analysis Guidance

The workgroup identified the "White House Guidance Document" *(Control of pathogens and vector attraction in sewage sludge*, EPA/625/R-92/013, 1999 update) as being the most recent guidance on sampling and options for analysis. They were satisfied with the information provided in the sampling section of the document that calls for biosolids to be sampled prior to disposal. There are different sampling protocols for sampling Class A versus Class B biosolids, and the minimum frequency of sampling is based on the output of the sludge processing facility.

The workgroup felt that ideally there should be a standard, or preferred, method for testing biosolids for the purpose of split sampling and consistency. They discussed the use of the ASTM method as the current standard method of detection and concluded that this method appears to be adequate.

The workgroup members questioned the sufficiency of current minimum monitoring requirements (ranging from once per year to once per month based upon volume of biosolids used or disposed) and would like to encourage responsible authorities to do more than the minimum monitoring.

Recommendations were also made on the following sampling issues: Virus recovery procedures for all viruses (not just enteroviruses) should be validated to ensure that the extraction process from the sewage sludge works for these other viruses (especially if the indicator organism is changed); and Laboratory certification, or minimum training requirements, is also important, and more information should be collected on cost effectiveness of these programs for states (some of which already have certification programs for metals, organics, and other contaminants) and commercial laboratories.

Matrix Effects

Regarding matrix effects on virus recovery efficiencies, the workgroup was concerned with how components of the biosolids sample could affect virus extraction. They recommended that extraction methods be tested for each virus in each form of Class A and B biosolids to find out what effect the matrix (e.g., compost, lime stabilized or alkaline treated biosolids; biosolids amended soils) has on the analysis.

Processing/Control Technologies

The workgroup first identified the following potential stressors as important to destroying viruses and of possible importance in specific treatment processes: Heat; pH; Oxygen regime (aerobic versus anaerobic environment); Irradiation (UV and gamma); Biological factors; Ammonia; Other chemicals (e.g., lime); Desiccation; time; and Pressure.

The workgroup then examined the relative effect of each stressor on the viruses of concern. Drawing upon available data and professional experience, participants rated the stressors shown in Table 3 based on the relative resistance (on a 3-point scale with "1" = most resistant and "3" = least resistant) of viruses to each stressor. Consequently, it would appear from this exercise that adenoviruses and hepatitis A virus are generally the most resistant to inactivation by the stressors indicated in Table 3.

Prioritized List of Research Needs

The workgroup identified the following as research priorities for addressing viruses in biosolids (H = highest priority; M = medium priority, and L = lowest priority):

- Risk assessment on Class B biosolids and vectors (e.g., flies) for virus transmission (H).
- Evaluate potential risk of exposure, especially to workers, to bioaerosols and dust resulting from the production and land application of Class B biosolids (H).
- Risk assessment for accidental human exposure (of the public) to land-applied Class B biosolids, including situations where food crops are grown/harvested (H).
- Transport and fate of viruses in land applied biosolids (M).
- Studies to determine the adequacy of a 30-day waiting period for grazing following land application of Class B biosolids (M).
- Development of new indicators for viruses in biosolids and creation of a matrix of virus levels present in different types of biosolids, by source of sewage sludge and type of treatment (M).
- Virus sample processing for greater recovery of viruses from soil samples (L).
- Generate data on concentration of non-enteroviruses in raw and treated biosolids (L).

Table 3. Relative[a] Effectiveness of Some Stressors in Inactivating Viruses

Virus or Virus Group	Stressor				
	Heat	High pH	Low pH	Temperature	Irradiation
Adenoviruses	1	2	1	1	1
Enteroviruses	2	1	1	2	3
Rotaviruses	2	2	1	2	2
Hepatitis A Virus	1	1	1	1	3
Hepatitis E Virus	?	?	1	?	3?
Caliciviruses	1?	?	1	2	3?
Reoviruses	2	2	1	2	2
Astroviruses	2?	2?	1	2?	3?
Syncytial Respiratory Virus	?	?	1	2?	3?
Blood-borne Viruses	3	3	3	3	3
Rhinoviruses	3	3	3	3	3

[a] 1 = most resistant; 2 = medium resistance; 3 = least resistant. ? = more research needs to be done.

Viruses in Animal Manures

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Introduction and Background

Raw and treated animal manures containing fecal matter, urine and other excretions are of increasing concern in animal and human health and with respect to environmental contamination. More and more viruses of animals and humans, some of which are known to be or may be capable of cross-species transmission, are being discovered. The rapid and dramatic spread of some agricultural animal viruses, such as Foot and Mouth Disease Virus (FMDV) in Europe, has heightened awareness and concern (Note: Foot and Mouth Disease of animals is caused by an aphthovirus and differs from hand-foot-mouth disease in humans which is caused by coxsackievirus). In the US, manures are regulated with little direct attention to their content of viruses and other pathogens. Nationally, and in many states, the virological quality of manures is not controlled or regulated. Therefore, farmers or other producers have no specific guidance or management systems specifically intended to manage the virological or other microbial content of these products. In many animal manure management systems it is assumed that the material remains on the farm and that viruses and other pathogens are contained and die-off over time. This is because many animal waste management systems are designed as and referred to as "non-discharge" systems. In these systems, the animal manure typically is treated or stored and then applied to the land. Land application of the manure is at rates based on nutrient loading, typically based on either nitrogen or phosphorus. Therefore, it is possible that animal manures applied to land can still contain appreciable concentrations of viruses and other pathogens. Once these pathogens are land applied, there is an appreciable loss of control over their transport and fate, and there are increased risks of environmental contamination both on and off farms.

In contrast to treated manures and related fecal wastes, human biosolids must meet specific virological and other microbial criteria and standards, defined as either Class A or Class B (USEPA 1993). Class A biosolids must be below detection limits for enteric viruses and viable helminth ova per 4 grams of total solids and must have <3 MPN (Most Probable Number) of *Salmonella* per 4 grams of total solids (dried sludge solids). Class B biosolids do not have specific end-product quality standards for viruses, but they must meet a numeric limit for fecal coliform bacteria and their applications and end uses are restricted to protect public health and the environment. *(Editor's Note: See paper by J.B. Farrell, these proceedings, for a detailed discussion of Class A and Class B requirements under the 1993 40 CFR Part 503 regulations.)*

Until recently, little effort has been made to characterize the reductions of viruses and other pathogens in animal waste treatment processes and manure management systems. Furthermore, little has been done to determine if viruses and other pathogens persist in treated manure and enter the non-farm environment to contaminate water, land or air. While some animal manure treatment processes and management systems have been designed to operate at conditions capable of inactivating or removing viral and other pathogens (e.g., composting and other thermophilic biological processes), the extent to which they actually reduce viruses and other microbes has not been adequately studied and characterized. For other animal waste management systems based on treatment processes not specifically designed for extensive pathogen removal or inactivation, such as various mesophilic biological processes, the extent to which they remove or inactivate viruses and other pathogens either has not been studied or has been studied only to a limited extent.

Like human enteric viruses, viruses of agricultural animals are difficult to detect and quantify for infectivity. Because such detection requires the use of cell cultures or experimental animals, it is technically difficult and relatively sophisticated, slow to produce results and is costly. These techniques are not practical for routine monitoring and surveillance. For this reason, there is an interest in knowing if indicator viruses or other indicators of viral presence and reduction efficiency are present in animal manures and are capable of providing a more practical, convenient, rapid and less expensive approach to viral monitoring for treatment performance verification and determining if manure treatment and management systems contribute to viral contamination off the farms.

In this brief review, some of the important viruses likely to be present in animal manures are identified, and their removal and fate in alternative animal manure treatment processes and management systems are described. In addition, indicator viruses in animal manures also are considered with respect to their relative concentrations, their reductions by treatment processes and management systems and the extent to which they migrate off farms and contaminate surface and ground water and other media.

Viruses in Animal Manures

A variety of different viruses can be present in animal fecal wastes and manures. Especially important are a variety of enteric and respiratory viruses, including animal enteroviruses, rotaviruses, adenoviruses, hepatitis E viruses, caliciviruses, reoviruses, parvoviruses and other non-enveloped viruses. Some of the main viruses and virus groups of importance in animal manures are shown in Table 1. Many of the viruses listed here are important pathogens of their animal hosts, although some of them do not appear to cause severe illness, high mortality or decreased production. The significance of many of these viruses to human health is uncertain or unknown. Because many of these viruses are non-enveloped, they are relatively persistent in the environment and resistant to treatment processes. Enveloped viruses also can be present in animal manures at high concentrations, and they may persist for considerable periods of time in the manure and in treatment and storage processes.

Recently, hepatitis E viruses (HEV) of swine and possibly other animal species have become of increasing concern with respect to human health because these viruses are prevalent in swine and present in swine wastes. Swine HEV has been shown to be experimentally transmissible to primates and human HEV is infectious for swine. Furthermore, swine and human HEV strains are genetically very similar on a country or regional basis. For example, in the United States, human and swine HEV are very similar genetically and the same is true for the human and swine HEVs in Taiwan (Erker et al. 1999; Hsieh et al. 1999).

The animal viruses in Table 1 are primarily of concern to agricultural animal health and productivity. Animal diseases caused by some of these viruses are responsible for high morbidity and mortality and reduced food animal production. Many of the viruses listed are not known to infect humans or cause human illness. However, a few of the viruses in Table 1, notably the caliciviruses, rotaviruses, myxoviruses (orthomyxoviruses and paramyxoviruses) and HEVs, are capable or may be capable of infecting humans. On rare occasions some of them, notably orthomyxoviruses such as swine influenza virus, have caused human illness. The extent to which animal caliciviruses, rotaviruses and animal HEV strains pose risks to human health remains uncertain but appears to be low in terms of documented risks of severe illness. However, epidemiological investigations of the human health risks of these viruses are limited, and therefore, the extent of such risks remains uncertain.

Virus Detection in Animal Manures

Although viruses have been studied for their survival, transport and fate in manure treatment and management systems, much of what has been done has relied on spiking samples with high concentrations of test viruses to follow their fate. Little has been done to develop, evaluate and apply methods to detect naturally occurring animal enteric viruses in raw and treated manures. There are concerns that the efficiency of virus recovery from animal manures may differ for spiked viruses and naturally occurring viruses. This is because the naturally occurring viruses may be embedded in manure solids and therefore harder to extract from the manure. Viruses have been recovered from manures seeded with high concentrations by simple extraction or elution procedures, and typically, no attempt has been made to further concentrate or purify the viruses (Deng and Cliver 1992). For direct inoculation into cell cultures, viruses in extracts of swine slurry have been treated with fluorocarbon (solvent) to improve virus recoveries and reduce cytotoxicity to cell cultures (Turner et al. 1999b). It is assumed that animal viruses in animal manures can be recovered and concentrated by the same methods used to recover human enteric viruses in municipal biosolids, but this has not been systematically investigated or verified.

Table 1. Some Important Animal Viruses Potentially Present in Animal Manure

Virus or Virus Group	Taxonomic Group	Animal Hosts	Disease in Animal Hosts	Human Infection/ Human Disease	Transmission Routes	Presence in USA	Presence in Manure
Entero-viruses	Picornaviridae	Bovine, porcine, avian	Yes in some	No, but maybe	Fecal-oral and respiratory	Yes for some, no for others (FMDV)	Yes
Caliciviruses	Caliciviridae	Bovine, porcine, avian	Yes in some	No, but maybe	Fecal oral and respiratory	Yes, some	Yes or probably
Reoviruses	Reoviridae	Wide host range for some	Some	Some yes, infection/ No illness	Fecal-oral; respiratory?	Yes, some	Yes
Rotaviruses	Reoviridae	Found in many animals	Yes, some	No, but maybe for some	Fecal oral; respiratory?	Yes, some	Yes
Adeno-viruses	Adenoviridae	In many animals	Yes, some	Unknown	Fecal-oral and respiratory	Yes, some	Yes
Herpes-viruses	Herpesviridae	In many animals	Yes, some	Unknown	Respiratory	Yes, some	Yes
Myxoviruses	Orthomyxoviridae and Paramyxoviridae	In many animals	Yes, some	Yes, some; No, others	Respiratory	Yes, some	Yes
Pestiviruses	Pestiviridae	In many animals	Yes, some	No,	Fecal-oral and respiratory	Yes, some	Yes, some
Corona-viruses	Coronaviridae	In many animals	Yes, some	No	Respiratory	Yes	Yes
Hepatitis E Virus (HEV)	Uncertain	Swine, rats, chickens, maybe others	Yes, but mild effects	Maybe	Respiratory and enteric?	Yes	Yes

"?" = Unknown

Bacteriophages of fecal bacteria present in animal manures are considered candidate indicators for the presence of viruses in manure, viral reduction by manure treatment and management systems, and viral contamination of manure origin in water and other media at animal farms. Of the bacteriophages present in animal feces and manures, the bacteriophages that have received the most attention as indicator viruses are the somatic and male-specific coliphages. These are the same types of bacterial viruses that have received similar attention as enteric virus indicators in human fecal waste, sewage and biosolids. Therefore, the extent to which coliphages are reliable indicators of human enteric viruses in treated biosolids and in environmental media impacted by biosolids is relevant to both animal and human fecal waste sources.

A number of methods to recover and detect somatic and male-specific coliphages in water, wastes and foods have been developed and evaluated over a period of several decades. Studies in the author's laboratory indicate that these methods can be readily applied to the recovery and detection of coliphages in animal manures and related samples (Hill and Sobsey 1998). That is, coliphages can be direct plated by quantal enrichment assays or single or double agar layer plaque assays. Alternatively, coliphages can be extracted or eluted from manure solids with beef extract or other eluent solutions. These eluent or extraction solutions can then be either assayed directly or the viruses in them can be further concentrated by polyethylene glycol precipitation and then assayed for infectivity by conventional plaque assay or quantal enrichment methods (Hill 2001).

Factors Influencing the Persistence and Fate of Agricultural Animal Viruses under Different Environmental Conditions

A variety of physical, chemical and biological factors can influence the persistence and stability of viruses in animal waste treatment and management systems. Some of the key factors are listed in Table 2. Virus survival in animal manures is probably most directly influenced by temperature, pH (either very high or very low levels), microbial activity, ammonia and indirectly by solids-association and other physical conditions of viruses (aggregation, encapsulation or embedding, etc.). Differences in the values of or conditions for these variables have been shown to dramatically influence virus survival in manures, biosolids and other matrices. However, it is not possible to rank these factors for their effects on virus survival, because the nature and magnitude of their effect depends on the actual level or state of the factor and the levels or states of the other factors.

Temperature and Thermal Effects

The persistence of both non-enveloped and enveloped viruses has been examined in liquid and dried animal wastes under a number of different conditions. Even enveloped viruses can be relatively persistent in animal manure. One of the more persistent animal viruses is Aujeszky's disease virus, also called

Table 2. Factors Influencing Virus Survival in Animal Manures and Biosolids

Factor	Effects
Physical	
Heat or thermal effects	Increasing inactivation at higher temperature; pasteurization
Desiccation or drying	Increased inactivation at lower moisture content or relative humidity
Aggregation	Clumping protects viruses from inactivating agents
Encapsulation or embedding	Viruses within membranes or larger particles are protected from inactivation
Chemical	
Hydrogen ions; pH	Viruses survive best near neutral pH and worst at pH extremes
Ammonia	NH_3 has virucidal activity; manifest at higher pH (>pH 8)
Enzymes	Proteases and nucleases contribute to virus inactivation
Biological	
Microbial activity	Biological treatment and microbial activity/metabolism in soils, sediments, water; several contributing mechanisms
Proteolytic activity	Proteolytic enzymes inactivate/denature virion proteins
Microbial predation	Engulfment, ingestion, etc. by protozoa, helminths, etc.

Pseudorabies Virus, which is a herpesvirus with a wide animal host range. The survival of the enveloped Aujeszky's disease virus in pig slurry was investigated during anaerobic storage at 5, 20, 35, 40, 45, 50 and 55°C using 100-ml laboratory models simulating the conditions in slurry tanks during winter and summer seasons and during anaerobic digestion in batch reactors. Inactivation rates increased with increasing temperature. Virus was inactivated at 5 and 20 °C in 15 weeks and 2 weeks, respectively. At 35°C (mesophilic conditions) the virus was inactivated in 5 hours, and at 55 °C (thermophilic conditions) no virus could be detected after 10 minutes. Although Aujeszky's disease virus was capable of persisting for considerable periods (days) in swine fecal wastes at mesophilic and lower temperatures, it was inactivated rapidly at a thermophilic temperature of 55°C (Botner 1991).

In another study, the persistence of a bovine enterovirus (BEV) (ECBO-virus strain LCR-4) and a field isolate of Aujeszky's disease virus was determined in liquid cattle manure stored at temperatures of 4 and 20°C for up to 26 weeks (Biermann et al. 1990). On the day of inoculation each sample had a titer of 5 $\log_{10}$ ID50/ml. Aujeszky's virus inactivation was >5 $\log_{10}$ after 16 weeks at 20°C but only 3.25 $\log_{10}$ after 26 weeks at 4°C. Enterovirus inactivation at 20°C was only 2 $\log_{10}$ after 26 weeks. Therefore, as expected, the non-enveloped (BEV) virus was more persistent than the enveloped virus (Aujeszky's virus).

The survival of the bacterium *Salmonella anatum*, Pseudorabies Virus (PRV), and porcine reproductive and respiratory syndrome virus (PRRSV) in swine slurry was studied at temperatures of 4, 25, and 37 °C and at pH 4.0, 7.0, and 10.0 (Ajariyakhajorn et al. 1997). These microbes survived longest at 4°C and pH 7, with *S. anatum* surviving longest at 56 days and PRV and PRRS viruses inactivated in 8 and 14 days, respectively. These results suggest that some viruses are not more persistent than bacteria under certain conditions in animal manures.

Most studies examining virus inactivation or persistence have been done under laboratory conditions, which raises concerns that they may not be representative of conditions in the field. Therefore, the persistence of five animal viruses, representing picorna-, rota-, parvo-, adeno-, and herpesviruses, and the coliphage f2 was determined in the field by exposing the viruses to different animal wastes using a filter sandwich technique (Pesaro et al. 1995). This method attempts to model the natural state of virus adsorption onto or incorporation into suspended solids, which may prolong virus survival. The "filter sandwiches" consist of viruses adsorbed to nylon filters that are sandwiched between layers of polycarbonate (PC) filters. The outer PC filters of the sandwich are either porous (15 nm in diameter) to allow passage of solutes or poreless (solid) to allow no passage of solutes, Using both types of PC filters, it was possible to differentiate between overall virus inactivation (from temperature and antiviral chemicals diffusing into the sandwich) and the effect of virucidal agents that act through temperature effects only (solid PC membranes). The virus-filter sandwich set-ups can be placed in manure storage or treatment systems in the field in order to study virus survival under the realistic conditions of actual field operations. Depending on ambient temperature, pH, and type of animal waste, time in days required for a 90% virus titer reduction varied widely, ranging from less than 1 week for herpesvirus to more than 6 months for rotavirus. Virus inactivation was faster in liquid cattle manure, a mixture of urine and water (pH > 8.0), than in semiliquid wastes that consisted of mixtures of feces, urine, water, and bedding materials (pH < 8.0).

It was concluded that unidentified virucidal agents that permeated poreless PC membranes contributed substantially to virus inactivation. Also, substances that protect rotavirus and possibly other viruses from inactivation also appear to be present in animal wastes. These apparently protective substances were not specifically identified in this study, but they are hypothesized to be similar to the previously identified detergents that thermally stabilized rotaviruses and other viruses in sludge (Ward and Ashley 1978; 1980). Overall, the results of the study indicate that viruses contained in manure may persist for prolonged periods of time if stored under nonaerated conditions. Therefore, virus persistence at times of land application may lead to environmental contamination. In summary, viruses in manures are inactivated more rapidly at higher temperatures and extreme pH levels. At moderate and low temperatures and at intermediate pH levels (pH 5-9), viruses can persist for considerable periods of time that may range from weeks to months and perhaps even years in the case of the most persistent viruses. Evidence of virus persistence in excess of a year in environmental media was obtained in soil column studies containing spiked viruses (Meschke 2001).

Proteolytic Activity Associated with Bacteria

The biological activity associated with proteolytic bacteria in animal wastes appears to contribute to virus inactivation (Deng and Cliver 1992; 1995). Antiviral bacterial activity was demonstrated in studies on the persistence of poliovirus type 1 (PV1) in mixed septic tank effluent and swine manure slurry and

by several bacterial cultures isolated from swine manure slurry. In field experiments, PV1 was inactivated more rapidly in mixed waste than in a control suspension of Dulbecco's phosphate-buffered saline (PBS). Times in days for 90% reduction of virus titer were 19–30 days for the mixed waste and 52–56 days for the PBS control. Virus inactivation in the mixed waste was temperature dependent. Comparing virus inactivation in raw mixed waste, autoclaved mixed waste, and bacteria-free filtrate of raw mixed waste suggested a role for microbial activity. At 25°C, 90% inactivation took 6.8 days in mixed waste, 11.2 days in autoclaved mixed waste, and 10.5 days in a bacterium-free filtrate of raw mixed waste. At 37°C, the corresponding values were 1.3, 3.9, and 3.1 days, respectively. At both temperatures, the differences in virus inactivation among the wastes were statistically significant. Three bacterial isolates caused virus inactivation in autoclaved mixed waste. This inactivation was prevented by protease inhibitors, suggesting an antiviral role for proteolytic enzymes produced by bacteria in the waste. The slower rates of virus inactivation in autoclaved waste could be due to destruction of antiviral bacteria and their products as well as destruction of other heat-labile constituents. The intermediate inactivation of viruses in bacteria-free filtrates of raw waste (compared to raw waste or autoclaved waste) could be due to the absence of antiviral bacteria but the continued presence of heat-labile antiviral chemicals, as well as the destruction by autoclaving of heat-labile chemicals that are protective of viruses.

In a subsequent study on the persistence of hepatitis A virus (HAV) in mixtures of septic tank effluent (STE) plus dairy cattle manure slurry (DCMS) and in mixtures of STE plus swine manure slurry (SMS), HAV was consistently inactivated more rapidly in the two types of mixed wastes than in STE alone or in a PBS control (Deng and Cliver 1995). At 5°C, 90% reduction of virus titer occurred in 35 days for the mixed STE and DCMS, 48 days for the mixed STE and SMS, 58 days for STE, and 217 days for the PBS control. At 22°C, the times for 90% inactivation were about 23, 17, 35, and 90 for the four test media, respectively. As in previous studies with PV1, HAV inactivation in the mixed wastes was partly related to microbial activity. In mixed STE and DCMS, 90% inactivation at 25°C occurred in about 8 days for raw mixed wastes, 15 days for autoclaved mixed wastes, and 10 days for bacteria-free filtrate of raw mixed wastes. At 37°C, these corresponding values were 7, 10, and 7 days for these three test media, respectively. In mixed STE and SMS at 25°C, 90% HAV inactivation was seen in 8 days for raw mixed wastes, 14 days for autoclaved mixed wastes, and 9 days for bacteria-free filtrate of raw mixed wastes; at 37°C these values were about 7, 9, and 7 days, respectively.

Persistence of Agricultural Animal Viruses in Waste Treatment Processes

The persistence and fate of viruses in animal waste treatment processes and management systems have not been adequately characterized and quantified. Only limited studies have been reported and most have been laboratory studies. Most studies have attempted to quantify reductions of virus infectivity in animal manure slurries or mixtures of these with other constituents under controlled temperature conditions and maintenance of either aerobic or anaerobic conditions. Studies on the fate of viruses after land application of animal manures or biosolids have not been reported (or at least could not be readily found in the peer-reviewed literature). Some of the main factors influencing virus reductions in animal manure treatment processes and the estimated virus reductions by these processes are summarized in Table 3. The term reduction includes virus inactivation (loss of infectivity) as well as physical removal of viruses. Some processes cause primarily virus inactivation, e.g., thermophilic processes. Others cause both inactivation and physical removal, such as many of the mesophilic biological processes, e.g., such as lagoons and constructed wetlands. Estimates of virus reduction are uncertain and are based on limited laboratory studies or pilot field studies and only with a few viruses, including indicator viruses (primarily coliphages). More specific information from individual studies reported in the literature is presented in the text following the table.

Drying and Desiccation

Enteric viruses in manures, sludges and other media are susceptible to drying, but only if very low moisture levels are achieved (<20%). Dewatering of raw sludge to <6 to 7% moisture content was shown to effectively inactivate human poliovirus, coxsackievirus and reovirus (Ward and Ashley 1977). Inactivation was due to disruption of the viral capsid with consequent release of nucleic acids. However, other studies have shown that viruses can persist in relatively dry soils for long time periods (Straub et al. 1993). Therefore, other factors in the suspending medium or matrix besides loss of water, such as detergents and ammonia, also may contribute to virus inactivation. Insufficient data are available for comparison of the survival of different types of viruses under dry conditions in different media, as only some virus groups have been studied in certain media.

Heat and Thermophilic Treatment Processes

Thermophilic treatments, including thermophilic digestion and pasteurization, have been shown to inactivate a number of animal enteric viruses. A bovine enterovirus and a bovine parvovirus seeded into liq-

Table 3. Summary of Animal Waste Treatment Processes and Virus Reductions

Treatment Process	Estimated Virus Reduction (log_{10})
Physical	
Heat/Thermal Processes	
Mesophilic	Typically, 1-2
Thermophilic	Typically, >4
Freezing	Variable
Drying or desiccation	Typically >4 at <1% moisture; Typically <1 at >5% moisture
Gamma Irradiation	Typically >3 log_{10}
Chemical	
High pH (>11)	Virus inactivation at high pH, e.g., alkaline/lime stabilization; typically, >3
Low pH (<2 to <5)	Virus inactivation at low pH; acidification: Typically, <2
Ammonia	Virus inactivation at higher pH where NH_3 predominates
Biological Processes	
Aerobic, mesophilic	Typically 1-2
Aerobic, thermophilic (composting)	Typically >4
Anaerobic, mesophilic	Typically 1-2
Anaerobic, thermophilic	Typically >4
Silage treatment, mesophilic	Variable
Land application	Highly variable and largely unknown; potentially high

uid cattle manure were rapidly inactivated by anaerobic digestion under thermophilic conditions at 55°C, but the same viruses survived for up to 13 and 8 days, respectively, under mesophilic conditions (35°C) (Monteith et al. 1986). The enterovirus was inactivated in digested liquid manure heated to 70°C for 30 minutes, but the parvovirus was not completely inactivated by this treatment (Monteith et al. 1986). Neither the enterovirus nor the parvovirus survived composting for 28 days in a thermophilic aerobic environment when seeded into the solid fraction of cattle manure. It was concluded that, of the procedures tested, only anaerobic digestion under thermophilic conditions was a reliable method of viral inactivation that ensured the safety of single cell protein derived from biological treatment of animal manure for feeding to livestock. Composting appeared to be a suitable method for the disinfection of manure for use as a soil conditioner.

The reductions of porcine parvovirus (PPV), bovine enterovirus (BEV) and fecal enterococci were measured in biogas continuous reactors for manure and manure supplemented with household waste at 35°C and 55°C and also in batch reactors at 70°C (Lund et al. 1996). Physiological saline was used as a control. Heat inactivation was the predominant inactivation factor at 70°C, but other factors, such as ammonia and possibly hydrogen sulfide and volatile fatty acids, also had virucidal and bactericidal effects. Inactivation rates were the highest for BEV, lower for fecal enterococci and the lowest for PPV. For fecal enterococci, the recommended 4-log_{10} minimum reduction was obtained in 300 hours at 35°C and in 1-2 hours at 55°C. In continuously fed reactors, reduction rates were initially high but then declined. For PPV, a 4 log_{10} reduction was achieved in 11-12 hours at 55°C initially (0-4 hours) and 54 hours thereafter (4-48 h). For 4 log_{10} reduction of bovine enterovirus, an RT of 23 hours was necessary at 35°C and < 0.5 hours at 55°C. Fecal enterococci were a good indicator for inactivation of enteroviruses and other more heat sensitive viruses, especially under thermophilic conditions. Parvovirus is useful to characterize virus inactivation kinetics at 50–80°C, due to its extreme thermal resistance. However, in establishing targets for treatment reactors it may be more reasonable to use a less resistant virus, such as a reovirus or picornavirus, which better represents the pathogenic animal viruses.

Heat treatment with combinations of temperatures and times was evaluated for inactivation of African swine fever (ASF) and swine vesicular disease (SVD) viruses in pig slurry (Turner and Williams 1999). ASF

virus (ASFV) was less resistant to both methods than SVD virus (SVDV). In slurry from one source, ASFV was inactivated at 65°C within 1 min, whereas SVDV required at least 2 min at 65°C. However, it was found that thermal inactivation depended on the characteristics of the slurry used. Thermal inactivation of the same two viral pathogens, ASFV and SVDV, in pig slurry also was studied in a pilot scale treatment plant (Turner et al. 1999). The plant treats pig slurry continuously at a rate of up to 100 liters/hour, heating the slurry to maintain at least 99.99% of it at the required temperature for a minimum period of 5 min, and then recovering the heat to raise the temperature of the incoming slurry. SVDV was inactivated in pig slurry to below detectable levels with an alkaline pH (pH 7.5 to 8, as is usually the case) at a temperature of 50–55°C. In acidified slurry (pH 6.4), inactivation occurred between 55–60°C. The difference in inactivation temperatures was probably due to the presence of free ammonia in the unacidified slurry. ASFV was inactivated by operating the plant at a temperature of 53°C at a pH of 8.

Decontamination of pig slurry containing foot and mouth disease virus (FMDV), Aujeszky's disease virus (ADV) and classical swine fever virus (CSFV) by thermal inactivation has been studied recently (Turner et al. 2000). Laboratory-scale experiments showed that FMDV, ADV and CSFV were heat inactivated in slurry within 3 min at 67°C, 3 min at 62°C and 3 min at 60°C, respectively. In Glasgow Eagle's medium, inactivation of the same viruses was achieved within 5 min at 67°C, 4 min at 65°C and 2 min at 65°C, respectively. At pilot scale, FMDV was heat inactivated at 66°C in water and 61°C in slurry, ADV at 61°C in water or slurry and CSFV at 62°C in water and 50°C in slurry. It was concluded that treatment of pig slurry for the inactivation of emerging or exotic viruses is possible in a thermal pilot plant operating in continuous mode, thereby ensuring the safety of pig slurry following a disease outbreak.

In summary, thermophilic physical processes and thermophilic biological processes, such as digestion and composting, are capable of achieving considerable pathogen reductions (>99.9%) These reductions are usually much greater than achieved by mesophilic biological processes (see below). The higher temperatures in thermophilic digestion and other thermophilic biological processes, such as composting, are highly microbiocidal due primarily to temperature effects. Thermophilic digestion of manures and biosolids achieves 99.99–99.9999% or 4-6 $\log_{10}$ reductions of fecal coliforms and >3 $\log_{10}$ reductions of viruses (Berg and Berman 1980).

Silage Treatment

Enteric viruses can be inactivated by the microbiological activity of various mesophilic or ambient temperature treatment processes. However, the rate and extent of virus reduction depends on the type of virus and the nature of the treatment process. When single cell protein seeded with bovine enterovirus or bovine parvovirus was ensiled with cracked corn, the enterovirus was inactivated after a period of 30 days, while the parvovirus survived for 30 days in one of two experiments (Monteith et al. 1986).

Alkaline Treatment

Alkaline treatment by addition of different concen-trations of NaOH or $Ca(OH)_2$ in combination with different times at 4°C and 22°C was examined for inactivation of African swine fever virus and swine vesicular disease virus (Turner and Williams, 1999). Addition of 1% (w/v) NaOH or $Ca(OH)_2$ inactivated ASFV within 150 s at 4°C, while 0.5% (w/v) NaOH or $Ca(OH)_2$ required 30 min for inactivation. NaOH or $Ca(OH)_2$ (1% (w/v)) was not effective against SVDV at 22°C after 30 min, but 1.5% (w/v) NaOH or $Ca(OH)_2$ inactivated SVDV at both 4 and 22°C. At higher alkali concentrations or temperatures, ASFV and SVDV inactivation was faster in slurry than in buffered medium.

Gamma Irradiation

Gamma irradiation has been suggested as a method to inactivate viruses in both human and agricultural fecal wastes and biosolids. The rate and extent of inactivation appear to depend on the type of viruses, the biosolids matrix, moisture content, temperature and other conditions. When an enterovirus and a parvovirus were seeded into single cell protein (the solids recovered by centrifugation of digested liquid manure), and dosed with 1 Mrad of gamma irradiation, the enterovirus was completely inactivated, but the parvovirus was only partially inactivated (Monteith et al. 1986).

Mesophilic Biological Processes

Most mesophilic biological treatment processes reduce pathogens by about 90–99% or 1-2 $\log_{10}$. For example, anaerobic lagoon treatment, a current BMP for swine manure in some states, produces microbial indicator (bacteria and viruses) and pathogenic bacteria (*Salmonella* spp.) reductions of about 90-99% (Hill and Sobsey 1998) in a single-stage lagoon. However, spores of the anaerobic bacterium *Clostridium*

perfringens are less extensively reduced (about 80%). In two-stage lagoon systems pathogen reductions of 99–99.9% are possible, including for *Salmonella* (Hill 2001). Additional pathogen reductions can be expected with additional lagoons in series. Pathogen reductions in swine lagoons show some seasonal effects, with enteric microbe reductions being higher during warmer months than during colder months. This is probably because pathogen die-off and the activities of biological processes are greater at higher temperatures. Other mesophilic biological manure treatment and storage methods have also been studied for pathogen reductions. Constructed wetlands have been shown to reduce enteric microbes (bacteria and coliphages) in anaerobic lagoon effluent by 90–99% (Hill and Sobsey 1998; 2001). Aerobic biofiltration has been found to reduce microbial indicators by about 90-99% (Hill et al. 2002). Anaerobic mesophilic digestion also reduces pathogens in animal manures. Overland flow achieves only 60–75% reduction of enteric bacteria and viruses (coliphages) (Hill and Sobsey 1998).

Transmissible Spongiform Encephalopathic (TSE) Agents or Prions

Transmissible spongiform encephalopathies (TSEs), sometimes known as prion diseases, are fatal degenerative brain diseases which occur in humans and certain other animal species. Examples of these diseases include Creutzfeldt Jakob Disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, and scrapie in sheep. All are caused by prions, which are unique protein agents with the ability to alter amyloid proteins in the brain through a type of catalytic activity. High concentrations of prions are found only in neurological tissue and the levels of prions in other tissues or excretions, including animal manures, are likely to be very low. In Europe, BSE in meat caused human disease, leading to the mass destruction of infected animal herds. The risk of human infection appears to depend on the animal source of the agent, species-related susceptibility, and the dose of the agent. Prions are highly resistant to conventional chemical and physical disinfection methods. They are not inactivated by formalin, ethylene oxide, autoclaving at conventional times and temperatures (e.g. 121°C for 15 minutes) or high doses of ionizing and UV irradiation. The infectivity of prions persists for long periods in the environment (Brown and Gajdusek 1991). Fortunately, prions are not highly contagious. Except for scrapie in sheep, prions do not appear to spread from an infected host to an uninfected one by normal contact. The risks of TSE transmission from water and other environmental media appear to be very low, based on a recent quantitative risk assessment (Gale et al. 1998). This is because the oral ID_{50} for humans (based on the oral ID_{50} for mice) is high (1 g of BSE-infected bovine brain or about 10^{13} BSE prion molecules). BSE infectivity is likely to remain bound to particulates in the aquatic environment, and through dispersion and dilution of BSE prions, consumption of drinking water, bathing water and other environmental media would result in exposure to minute sub-fractions of an ID_{50}.

Summary, Conclusions and Recommendations

A variety of viruses, both enveloped and non-enveloped and representing many taxonomic groups, are likely to be present in animal manures. Some of the important viruses potentially present in animal manures are not present in the United States, but there is growing concern that non-endemic viruses may be introduced either accidentally or deliberately into United States animal populations. Animal viruses not endemic in the United States include African Swine Fever Virus, Akabane, Classical Swine Fever Virus, Foot and Mouth Disease Virus, Louping Ill Virus, Lumpy Skin Virus, Rift Valley fever, Rinderpest, Vesicular Stomatitis and Viral Hemorrhagic Disease of Rabbits. New viruses, such as Nipah Virus, continue to be discovered in agricultural animal populations and the host ranges of these viruses are uncertain. There are concerns that some of these recently discovered animal viruses do infect or may be able to infect humans or that they have the potential to recombine with human viruses and produce new virus strains capable of infecting humans. Particular viruses of concern in this regard are hepatitis E virus, caliciviruses and orthomyxoviruses (influenza viruses). A current list of non-endemic viruses of concern for animals in the United States can be found in Foreign Animal Diseases "The Grey Book" http://www.vet.uga.edu/vpp/gray_book/pdf/).

The reductions of viruses by animal waste treatment processes and animal waste management systems have not been extensively studied. Therefore, there are considerable uncertainties about the extent to which animal viruses survive waste treatment processes, are released into the environment and are available to be transported off farms. Off farm contamination can potentially occur inadvertently, such as unplanned and uncontrolled release by runoff, aerosolization or infiltration into soils and ground water, or when manure residuals are transported off of farms to be land applied, marketed or used for other beneficial uses. The extent to which these and other emerging animal viruses are removed and inactivated in various waste treatment processes and management systems is uncertain and needs further investigation.

The reductions of animal viruses by some animal waste treatment processes have been determined in laboratory and pilot scale field studies. In general, thermophilic processes, such as pasteurization, thermophilic digestion and composting, are capable of producing extensive (>4 $\log_{10}$) virus inactivation, thereby resulting in treated residuals that are likely to contain only low virus concentrations. Further studies are recommended to better characterize virus inactivation in thermophilic processes for manure treatment and to define the optimum conditions to achieve extensive virus reductions.

Drying of some animal manures is a widely practiced management approach in some places. However, little is known about the extent to which viruses are inactivated in manure drying processes or during dry storage because there have been few if any studies to document their effectiveness. Desiccation or drying to very low moisture levels (<1%) has been shown to result in extensive (>4 $\log_{10}$) human enteric virus inactivation in municipal biosolids and in soils. Therefore, studies are recommended to determine the rate and extent of animal virus inactivation in drying and desiccation processes for animal manures.

Most mesophilic biological treatment processes for animal manures are not likely to reduce virus levels by more than 1-2 $\log_{10}$ or 90–99%. Therefore, treated manures or biosolids from such processes may still contain high concentrations of viruses. The fate of these viruses in subsequent management operations, such as land application or prolonged storage, is uncertain and has not been adequately determined. Therefore, further studies on effectiveness of mesophilic treatment processes in reducing enteric viruses and on the fate of viruses in these post-treatment management processes are recommended.

Chemical treatments of animal manures are typically by lime or other alkaline treatment. Such treatment is widely practiced for municipal biosolids but less so for animal wastes. Alkaline stabilization for virus inactivation has been highly effective in municipal biosolids and promising results have been obtained when it has been applied to animal manure solids. Therefore, further studies are recommended to better characterize virus inactivation by alkaline treatments of animal manure solids with respect to solids composition, pH and storage and handling conditions.

The ultimate fate of viruses in animal manure management systems remains uncertain, especially for large scale, multi-stage systems involving treatment or storage followed by land application at production facilities with large numbers of animals and minimum acreage (confined animal feeding operations). Because of the magnitude of animal wastes generated by these facilities and the potentially high pathogen loadings that can result if the treated manure residuals still contain high virus concentrations, further investigation of the fate of viruses in these systems and their surrounding environments is recommended.

Definitive methods to recover and detect viruses in animal manures and their treated residual solids have not been reported, especially for emerging viruses, such as HEV and caliciviruses. Therefore, the extent to which these viruses are removed or inactivated in animal waste treatment processes or management systems remains uncertain due to uncertainty associated with the virus recovery and detection methods. The development, evaluation and application of reliable, sensitive and affordable methods to recover and detect animal viruses in animal manures and their treated residual solids are recommended.

In principle, methods are available to recover and detect indicator viruses, such as somatic and male-specific coliphages, in animal manures and their treated residual solids. However, these methods have not been adequately verified and collaboratively tested in these types of samples. Such verification and performance characterization studies are recommended. Also recommended are comparative studies on the removal, inactivation and fate of indicator viruses and animal viral pathogens in manure treatment processes and management systems. If such studies show that the indicator viruses reliably reflect or predict the responses of the animal enteric viruses to manure treatment and management processes, it then becomes possible to use them in practical, rapid and affordable monitoring and surveillance activities to assess treatment process and system performance and the virological quality of the treated residuals.

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Viruses–Animal Wastes Workgroup: Discussion Summary, Conclusions and Recommendations

This workgroup focused on viral pathogens that may be present in animal manures. It considered viruses that affect human health and viruses that affect animal health. An exercise was completed attempting to indicate the hazard to human/animal health associated with several viruses.

Participants in this workgroup were: Mark Sobsey, Leader; Shay Fout, Facilitator; Judy Akkina; Jesus Martinez Almela; Donna Francy; Paul Gale; Sagar Goyal; Mike Johnson; Jamie Jonker; Michael McLaughlin; Laurel Staley; Nina Sweet; and Matias Vanotti.

Organisms

The workgroup created a list of viruses of concern, based on potential human or animal health hazard.They are presented below with general comments from the dialogue about each type of virus:

- Hepatitis E virus (HEV): Found in swine (rats can be vectors)
- Reoviruses: Type 3 was identified as a priority
- Rotavirus groups A and B: Bovine and porcine viruses
- Caliciviruses: Bovine, porcine virus with implications for animal health; may transfer from one species to another in the wild. These viruses are highly transmissible. They are also numerous. Caliciviruses are the most dominant viruses in pigs. Type h1 used to be predominant. Recently h3 has been isolated and it is now evident in hog waste. This could be a concern for animal health.
- Myxoviruses: Influenza viruses found in swine and poultry
- Enteroviruses: Insects are potential vectors (mosquitoes should be considered); include waterborne pathogens and also those that emerge during drought
- Less important viruses: Coronaviruses, Parvoviruses, Paramyxoviruses, Hepadnaviruses, Retroviruses and vector-borne viruses.

Although prions are not viruses, it was decided to include them in the viruses workgroup in order to raise awareness to them. Prions are proteinaceous infectious particles that are hardy and difficult to destroy. One assumes no destruction by standard manure treatments used on farms. Three known prion diseases of livestock are: scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in deer and elk (WOAH website; USDA website). Scrapie occurs in most countries of the world, including the United States. Australia and New Zealand are known to be free of scrapie. BSE has primarily been found in Europe. *(Editors note: BSE has recently been confirmed in isolated incidents in Japan, Canada and the United States.*) CWD occurs in localized areas of the United States and Canada. Due to species and disease variations, an assessment of transmission risk from manure to animals should be disease and animal specific (ie. scrapie in sheep, BSE in cattle, CWD in deer).

In cattle with BSE, infectivity has been found in the brain, spinal cord, trigeminal ganglia, retina, dorsal root ganglia, bone marrow, and distal ileum, but not in manure (Jones et al., 1996; Wells et al., 1998; UK DEFRA website). Use of compost which included dead cattle or cattle parts (neural tissues, etc.) on land grazed by cattle would be a possible transmission concern. Similarly, compost which included dead sheep, and used where sheep grazed, would be a possible transmission concern for sheep. The sheep scrapie pri-

on has been found more widely distributed in sheep tissues than the BSE prion in cattle tissues, including the brain, spinal cord, peripheral lymph nodes (e.g., tonsil, retropharyngeal lymph node, mesenteric portal lymph node, 3rd eyelid), ileum, spleen, bone marrow, pancreas, thymus, liver, peripheral nerves, placenta, and fetal membranes. Infectivity has not been found in blood clot, feces, heart, kidney, mammary gland, milk, ovary, saliva, salivary gland, seminal vesicle, testis, serum, skeletal muscle, thyroid, uterus, fetal tissue, bile, bone, cartilaginous tissue, connective tissue, hair, skin and urine (UK DEFRA website). The means of scrapie's natural transmission have not been fully defined. However, transmission is thought to be primarily via an oral route by ingestion of placental tissue, membranes or fluids, or through contact with feed, water or pasture contaminated with infectious tissues or fluids.

The group concluded that prions are an unlikely manure risk in the United States.

An attempt was made to prioritize the above viruses based on six factors that relate to the human health and environmental impact of each. The factors were: Occurrence in animals in the United States; Concentration of pathogens in manure; Likelihood of impacting human health; Amenability of the pathogen to reduction/removal by treatment; Survivability of the pathogen in the environment; and Infectious potential of the pathogen.

Table 1 shows the relative danger to human health posed by select viral pathogens of concern with respect to these six factors. Generally, for each group of viruses, the factors were scored as High, Medium, or Low. In some instances, supplemental information (shown in parentheses) is given on occurrence, human or animal health effects and infectious dose. The factors were scored by group consensus. In several instances, data are lacking or were unknown to the group and this is indicated by a question mark.

Editor's Note: Mark Sobsey summarized the significance of viruses in animal manures as follows: "The animal viruses in Table 1 are primarily of concern to agricultural animal health and productivity. Animal diseases caused by some of these viruses are responsible for high morbidity and mortality and reduced food animal production. Many of the viruses listed are not known to infect humans or cause human illness. However, a few of the viruses in Table 1, notably the caliciviruses, rotaviruses, ortho myxoviruses and hepatitis E viruses, are capable or may be capable of infecting humans. On rare occasions some of them, notably ortho myxoviruses such as swine influenza virus, have caused human illness. The extent to which animal caliciviruses, rotaviruses and animal HEV strains pose risks to human health remains uncertain but appears to be low in terms of documented risks of severe illness. However, epidemiological investigations of the human health risks of these viruses are limited, and therefore, the extent of such risks remains uncertain."

Table 1. Hazard Identification and Pathogen Categorization

Pathogen	Occurrence in U.S. Animals	Conc. In Manure	Human Health Effects	Manure and Treatability	Environmental Persistence	Infectivity*
Swine HEV	High	Medium	Low (?); Pregnant Women (?) (Human HEV)	?	?	High (low dose) – pig
Reoviruses	Medium	Medium	Low	Medium	Medium	High (low dose)
Rotaviruses Group A	High	High	High (human); ? (animal)	Medium	Medium	High (low dose)
Rotaviruses Group B	High	High	Low (human); ? (animal)	Medium	Medium	High (low dose)
Calicivirus	Medium	Medium	?	?	Medium/High	High (low dose)
Myxoviruses	High (pig, avian)	Medium/Low	Potentially High; Prevalence Low	High	Low	High (low dose)
Enteroviruses	Medium	Medium/High	Unknown ?; Probably Low	High	Low	High (low dose)

* No information on human infectivity risks except for enteroviruses; not known if animal infectivity data is predictive of human infectivity.
? = Data lacking or unknown to workgroup.

The discussion participants summarized the priority of the virus groups and prions in Table 2. The priority (high or low), the group, and the important host animal species are shown.

Table 2. Priority Classification of Animal Viruses and Prions in Manures

Priority Rating	Group	Host Animal Species
High	HEV	Swine (Rats)
	Reoviruses (Type 3 and others)	Many
	Rotaviruses (Groups A and B)	Many (esp. Bovine, Porcine)
	Caliciviruses	Many (esp. Bovine, Porcine)
	ortho Myxoviruses	Swine, Poultry (Others?)
	Enteroviruses	Bovine, Porcine (Others?)
Low	Herpesviruses, Coronaviruses, Parvoviruses, Paramyxoviruses, Hepadnaviruses, Retroviruses	Many
	Vector-borne viruses	Many
	Prions	Bovine, Ovine, Cervine (Others?)

Indicators

The workgroup considered a number of different indicators including coliphages (bacterial viruses), human enteric viruses (like Herpes A), and chemical indicators. Coliphages are potential indicators of viruses from manure contamination and of treatment efficacy. While coliphages are relatively consistent as indicators of fecal contamination, they are not necessarily good indicators of treatment effectiveness. For example, specific coliphages would not be good for predicting effectiveness of thermal processes because they are easily killed. Coliphages also die off rapidly at pH 4-4.5, and environmental stressors may influence their suitability as indicators. Although USDA and possibly others are doing some work on coliphages, there is a lack of information concerning their use as indicators of treatment effectiveness.

Animal viruses (those infecting humans and animals) have been used for methods development, determining treatment effectiveness, and as indicators of fecal contamination. Spiked animal viruses could be used to determine treatment effectiveness, but there are problems with this approach. Most animal viruses are difficult to grow; variability in their growth may limit the reproducibility of results, and there are no simple methods to work with them in model systems. There are also human health concerns with spiking animal viruses. Poliovirus type 1 was used in some earlier studies and became a model virus for obtaining a variety of environmental biology data. The vaccine strains of polio are capable of causing human disease and releasing potentially pathogenic viruses in the environment would obviously be problematic.

The workgroup concluded that chemical indicators (e.g., fecal sterols) could be used for source identification and tracking, but should fall toward the bottom of the list of ideal indicators because they cannot be used to indicate treatment efficacy or environmental persistence of viruses.

Table 3 presents the candidates that the workgroup believes are acceptable indicators of virus contamination from manures and of the effectiveness of manure treatment systems.

Ability To Assess Risks

Possible environmental health impacts and concerns of viruses in manures result from ground and surface water contamination. Public health risks include edible food crops eaten raw, residuals taken off farms for commercial use, and bioaerosols and airborne transmission of viruses. To assess the risks associated with viruses and animal manures, the following were addressed: the quantity and quality of data available; whether or not the data were from municipal biosolids or manures; the information presented in Table 1; and transmission pathways.

Table 3. Potential Indicators of Viruses in Manures and Manure Treatment Systems

Virus	Host	Advantages	Disadvantages
Enteroviruses	Beef, pork	Easy to grow; PCR available	Species-specific
Reovirus Type 3	Widespread	Easy to grow; PCR available	Modest growth rate

In assessing risk, it is essential to understand source receptor pathways for the stressor whose risk is being assessed. Considerable time was spent discussing transmission pathways for viruses in manures. In wastewater treatment systems, contaminants can leach into the water following land application. They can go directly from manures into water, thus providing a waterborne transmission pathway. Crops can become contaminated from the same sources, mostly from land application. Figure 1 depicts the source receptor pathways identified for viruses in animal manure.

Air sampling has provided some cause for concern about the potential for airborne transmission. Agricultural studies have been performed where municipal wastewater was applied with subsequent evidence of airborne transport to downwind sites (Camann et al., 1988). Manures have a much higher concentration of microorganisms than secondary effluents. Workers and consumers become exposed to pathogens by coming into contact with soils, crops, and treated wastewater. Animals also become exposed by coming into contact with crops and contaminated water.

The group reached the following conclusions concerning risk assessment:

- There is a paucity of actual data for risk assessment or risk management decision-making and actual practice. Much of the data is from studies on human viruses in municipal wastewater or sludge rather than from studies on animal manures.
- A major research effort to develop needed data for all aspects of risk assessment, improved management decisions, and regulatory decision-making is needed.
- A monitoring system for some of the public concerns that are connected with animal production activities should be developed.

Detection/Analytical Capabilities

See *Prioritized List of Research Needs* below.

Processing/Control Technologies

The workgroup discussed the effectiveness of various processing and control technologies in terms of the total $\log_{10}$ reduction of viruses achievable for certain animal wastes. Table 4 lists the processes considered with the approximate $\log_{10}$ reduction and animal systems that the technology can be applied to, along with comments, where appropriate, on retention time and temperature considerations. A question mark indicates that data are not available or the group did not know them.

Prioritized List of Research Needs

Though the workgroup would have liked their conclusions to be supported by literature references, they concluded from their dialogue that there is a paucity of actual data necessary for conducting risk assessment. A major research effort will be required to fill data gaps. Some of the research needs they identified for animal wastes and viruses include:

- Rapid detection methods and shorter analytical procedures for indicator organisms;
- Virus reductions in solids separation processes;
- Research to determine effectiveness of riparian buffers for preventing pathogen transfer/transport off farms and land application sites;
- Studies to determine adequacy of harvest and grazing restrictions for biosolids applications for the prevention of virus transfer and studies to determine appropriate restrictions for manure;
- More data addressing the fate and transport of bioaerosols from spray irrigation with wastewater/liquid manure and/or land application.

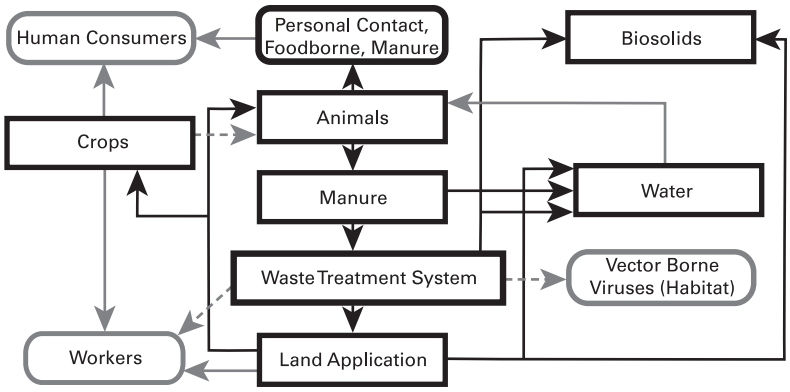

Figure 1. Source Receptor Pathways for Viruses in Animal Manure

Table 4. Virus Reduction in Waste Treatment Processes and Systems

Unit Process	Log_{10} Reduction	Animal System	Comments
Liquid Manure Processes			
Anaerobic Lagoons	1-2/ unit	Swine, dairy	Retention time in months
Aerated Lagoons	1-2/ unit	Swine, dairy	Retention time in months (bioaerosols?)
Anaerobic Digesters Mesophilic Thermophilic	 1-2/ unit 3->4/unit	 Swine, dairy Swine, dairy	
Constructed Wetlands	1-2/cell	Swine, dairy	Less effective in cold weather
Overland Flow	~50% in effluent	?	
Solids Separation, Physical	<1 (?)	All	
Aerobic Biological Processes	1-2 (?)	Swine	
Chemical Coagulation, Flocculation, Precipitation	1-2 (?)	All	
Alkaline Treatment	1-2 pH <11 3-4 pH >11	All	Must ensure high pH for all material
Dry Manure Processes			
Composting	4+	All	Time and temperature dependent; mixing/aeration important
Thermal Processes 55-60 C >60 C	 3-4 4+	All	Time and temperature dependent
Drying and Dry Storage	<1 at >3% moisture >3 at <1% moisture	All	Time dependent Moisture dependent (climate) A few viruses are not inactivated by drying or desiccation

? = Data not available or unknown to workgroup

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SECTION IV

Parasites in Biosolids/ Treated Sewage Sludge and Animal Waste

Concerns Related to Protozoan and Helminth Parasites in Biosolids and Animal Wastes

Parasites—Sludge/Biosolids Workgroup: Discussion Summary, Conclusions and Recommendations

Parasites—Animal Wastes Workgroup: Discussion Summary, Conclusions and Recommendations

Concerns Related to Protozoan and Helminth Parasites in Biosolids and Animal Wastes

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Introduction

The total population in the U.S. on November 1, 2000 was estimated to be 276 million persons (http://www.census.gov). Data on plumbing facilities (including flush toilets) were dropped from the census after 1990. At that time 101.1 million homes had complete indoor plumbing but 1.1 million homes still lacked complete plumbing. Public sewers have served a vast majority of America's homes since 1970 and most other homes have used a septic tank or a cesspool. Only a tiny percent had neither a public sewer, septic tank, or cesspool. In 1970, a number of states, primarily in the South (plus Alaska), had 10% or more of living units still using a privy, a chemical toilet, or facilities in another building, but by 1990, only Alaska still had a high percentage of such units. In addition to human wastes, animal wastes may be a significant source of parasitic pathogens. Only three species of domestic animals (other than cats and dogs) are present in the U.S. in significant numbers: 100 million cattle (35 million beef cows, 55 million steers, calves, and bulls, 10 million dairy cows); 100 million pigs; and 8.5 billion chickens (8 billion broilers, 0.5 billion layers). Sheep and horses number in the few to several million (http://www.usda.gov/nass/pubs/stathigh/2001/tables/lvstkindex.htm). Dogs and cats number about 50 million each (http://www.avma.org/press/pidemosb.asb), and their waste often enters either manure or biosolids through wastewater or runoff. Chickens carry no parasitic pathogens that are truly of concern relative to manure transmission to people.

Information is presented in this paper on parasites found in municipal biosolids or animal wastes and considered to be of concern to human health. Table 1 is a listing of those zoonotic pathogenic parasites that may pose a threat to persons in the United States. Included are two new and emerging pathogens, *Cyclospora* and the Microsporidia, and one long-known pathogen, *Toxoplasma gondii*, which became worrisome because of a waterborne outbreak in Canada (Isaac-Renton et al., 1998). Ignored are many pathogens that are unlikely to survive in the typical wastewater treatment system even if they were present, thus, no information is presented on parasites such as hookworms, schistosomes, or other flukes that require snail intermediate hosts. Animal pathogens that are infectious only to other animals, e.g., the coccidia of cattle, pigs, and swine, have been ignored because they do not pose threats to human health.

Parasitic Pathogens in Sludge/Biosolids and Animal Wastes of Concern to Human Health

The material below includes aspects of the biology and physical makeup of the parasites that are important for an understanding of how they behave in wastewater treatment systems and how they protect themselves from various inactivation processes. Also included are data about the effects of existing treatment modalities on the viability of these organisms. Finally, information is given about the organisms as it relates to how well various methods of detection may work in wastewater effluents and biosolids, the current state of the art in pathogen detection, and a few recommendations as to further research needs.

Table 1. Zoonotic Parasites Resistant to Treatment Processing of Sludge/Biosolids and Animal Wastes

Parasite	Potentially Present in	
	Sludge/Biosolids	**Animal Wastes**
Protozoa:		
Balantidium coli	Not Likely	Pigs
Cryptosporidium spp.	Yes	Cattle, Pigs, Dogs, Cats
Giardia spp.	Yes	Cattle, Pigs, Dogs, Cats
Entamoeba histolytica	Yes	No
Cyclospora	?	?
Toxoplasma gondii	Yes	Cats
Microsporidia	Yes	Cattle, Dogs, Cats
Helminths:		
Ascaris spp.	Yes	Pigs
*Baylisascaris procyonis**	Yes	Raccoons, Dogs
Trichuris spp.	Yes	Pigs
Toxocara spp.	Yes	Dogs, Cats, Cattle (Rare)
Taenia	Yes	Dog, Cats

*Not covered in the presentation or below but it was discussed by the Parasites/Biosolids Discussion Group. See Sorvillo et al., 2002 for a review.
? = Data not known.

Protozoa

Cryptosporidium parvum

I. Introduction to the pathogen

A. Summary of life history as related to transmission - *Cryptosporidium parvum* is an apicomplexan parasite of the mucosa of the small intestine of man and other mammals. The life history has been reviewed in depth (Fayer et al., 1997). Within the host, there are various obligate intracellular stages that function for the purposes of multiplication and sexual recombination. The stage that transmits this parasite between hosts is the product of sexual recombination and is called an oocyst. After the fusion of the gametes, a protective wall is formed around the cytoplasm of the fertilized zygote, and the resulting oocyst is apparently mechanically released from the parasitized host cell. The oocyst stage is transferred from host to host by fecal-oral contact or by the ingestion of the infective oocysts in the water or on contaminated foodstuffs. In the typical host, the infection is self limited, and the oocysts represent the termination of the infection process and the culmination of multiplicative and recombinatorial processes. However, it appears that some oocysts may excyst prior to leaving the body, and in hosts that are immunosuppressed, the host is infected by the sporozoite stages released from the oocyst. The disease that occurs in the infected host is a diarrhea due to the damage caused to the parasitized epithelial cells of the small intestine.

B. Potential pathogenic effects on humans or livestock - Infection results in diarrhea, dehydration, and possibly malnutrition. Infection can be life threatening in immune compromised persons. There is no FDA approved prophylaxis or chemotherapy.

C. Physical nature of the resistant stage - The oocyst of *Cryptosporidium parvum* is spherical and about 5 μm in diameter. The specific gravity of the oocyst has been determined to be about 1.085. The permeability of the oocyst wall has been previously examined with most of the work being directed at inactivating the infectivity of oocysts for the purpose of decontaminating drinking water. Typically, the mode of inactivation has not been considered. For example, oocysts were treated with seven disinfectants, including iodophore, formal saline, cresylic acid, sodium hypochlorite, benzylkonium chloride, sodium hydroxide, and ammonia (Campbell et al., 1982), but little attention was given to the mechanisms by which these disinfectants might be entering, excluded, or acting on the contained sporozoites. Other work has also examined

the effects of various disinfectants on *Cryptosporidium parvum* (e.g., Pavlasek, 1984; Blewett, 1989; Peeters et al., 1989; Korich et al., 1990; Holton et al., 1994; Holton et al., 1995), but none of these papers has considered the mechanism of action of the disinfectant on the oocysts. Thus, it is not known if the effects are due to changes in the barriers within the oocyst wall, the ability of the disinfectants to pass through the barriers in the oocyst wall where they then act directly on the contained sporozoites, or even if the effects of the disinfectants are due to their action on the sporozoites and whether or not the sporozoites have any capabilities of self preservation. In fact, while indirect evidence indicates that water is capable of moving across the barriers provided by the oocyst wall, this phenomenon is not well documented.

D. Structure of the resistant stage - The morphology of the oocyst wall of *Cryptosporidium parvum* and the presence of the suture has been described (Fayer et al., 1997). The wall has been described as 31.6 to 72.9 and 40 to 100 nm in thickness (Reduker et al., 1985; Uni et al., 1987, respectively). The outer layer, which is moderately electron dense, makes up about 20% of the entire wall. The inner layer consists of two distinct zones. The zone of the inner layer that is closest to the outer layer comprises another 20% of the total width of the wall and appears as an electron dense granular area. The inner zone of the inner layer comprises about 50% of the entire wall's thickness and is a slightly less dense granular layer very similar in structure, except for density, to the other zone of the inner layer. Between the outer layer and the inner layer is an electron lucent space that is 10% of the width of the oocyst wall; work by Scholtyseck (1973) has suggested that this space is very likely a fixation artifact. In unexcysted oocysts, a suture extends approximately one-third to one-half the way around the oocyst; in the area of the suture there is a thickening of the inner layer which extends further into the interior of the oocyst. The suture appears as a pair of parallel electron dense opacities in the inner layer of the oocyst wall. In tangential section, these opacities extend from the bottom of the thickened inner layer to where it meets the outer layer; and appear to be three times taller than they are wide. Overall the opacities in the inner layer which form the suture are oriented such that they appear like rails of the track-like suture which runs across one-half to two thirds of the oocyst's surface. It has been shown that during excystation, the two sets of rails separate much like the two sides of a zipper, although it is not clear whether the process begins at one end of the suture, at some point along the suture, or along the entire length of the suture.

E. Composition of the oocyst wall - There is almost nothing known about the structural components of the oocyst wall. The biochemical nature of the wall is unknown. From the work of Stotish et al. (1978) on the oocysts of *Eimeria tenella*, it has been generally assumed that the oocyst wall of a coccidian (the group to which *Cryptosporidium* belongs) contains somewhere around 67% peptide, 14% lipid, and 19% carbohydrate. However, there has been no work demonstrating that this is actually the case for *Cryptosporidium* or which of the layers observed ultrastructurally actually correspond to the different potential components of protein, lipid, and carbohydrate. The reality is that it is not known whether the external portion of the oocyst wall or the internal portion of the oocyst wall is the protective layer and/or semipermeable barrier. What is known is that antibodies can be produced to the oocyst that are capable of recognizing epitopes on the oocyst surface (LeChevallier et al., 1993). A recombinant protein associated with the oocyst wall that has high proportions of cysteine, proline, glutamine, and histidine, and distinct cysteine-rich regions of repetition has been characterized (Lally et al., 1992; Ranucci et al., 1993). Mitschler et al. (1994) reported the lipid composition of whole *C. parvum* oocysts without any distinction being made between oocyst wall and sporozoites. There does not appear to be any published information relative to the carbohydrate content of the oocyst wall.

F. Discussion of what is known about potential risk - Over 150 species of mammals have been reported to be infected with *C. parvum* based on microscopic observations of oocysts in feces (Fayer et al., 2000). Three Orders of mammals appear to be most susceptible to infection: artiodactyls, rodents, and primates, although most Orders are represented. Without supportive molecular data it is presumptive to accept that the 150+ species of mammals have been infected with the same species and genotype of *Cryptosporidium*. Without such information it is impossible to know which infected animals pose a risk for humans. Populations most at risk of acquiring infection are persons in day-care centers, persons using public recreational

waters, and immune compromised individuals. The latter group also are at greatest risk of life-threatening infections.

G. Prevalence rates in human and animal populations in the US - The actual prevalence in the human population is not known but over the past 10 years, hundreds of thousands of persons have become ill in outbreaks related to drinking water and recreational water (Fayer et al., 2000). From the standpoint of human health, the major genotypes of *Cryptosporidium* of concern are genotypes 1 (now known as *Cryptosporidium hominis*, Morgan-Ryan et al., 2002) and 2 (containing the original *C. parvum*) (Peng et al., 1997). Humans are the only source of genotype 1. Probably over 90% of neonatal calves become infected and excrete large numbers of genotype 2. The proximity of cattle to surface water and the likelihood of fecal runoff from fields and pastures raise concern that cattle are a major source of waterborne *Cryptosporidium* infections for humans. Few studies have determined the genotypes found in most other mammals.

H. Geographical distribution in the US - Ubiquitous and prevalent.

I. Number of stages produced by a single infection - For nonimmune persons and animals the infectious dose is low but may vary somewhat with different isolates. For humans the ID50 is about 100 oocysts of *C. parvum* genotype 2 (Dupont et al., 1995). Other strains of genotype 2 have produced ID50s ranging from about 10 to 1000 oocysts (Okhuysen et al., 1999) For neonatal cattle the infectious dose is also very low. Naturally infected and experimentally infected neonatal cattle can excrete 10^9 oocysts or more over a period of approximately 1 week (Nydam et al., 2001).

J. Relating known outbreaks to pathogens in wastewater or manure - The first major outbreak in a community surface water supply was associated with sewage contamination of the source water — the Carollton, Georgia outbreak involving an estimated 13,000 persons (Hayes et al., 1989). A number of outbreaks in the U.S., Canada and England have been attributed to sewage or animal manure contamination of the source water (Craun et al., 1998).

II. Suspected modes of transmission

A. Effluents - See Carollton, Georgia outbreak above (Hayes et al., 1989). In a study at the Montreal Urban Community wastewater treatment facility (primary treatment only, 2 billion gallons per day), the primary settling removed only 27% of the *Cryptosporidium oocysts* in the influent (Payment et al., 2001).

B. Biosolids - Only a small percentage of the oocysts are expected to be collected in the biosolids portion of a sewage treatment plant. As can be seen from the above cited work at Montreal (Payment et al., 2001), oocysts tend not to be well-concentrated by primary clarification. Also, the specific gravity of the oocysts being rather close to that of water makes it difficult for the oocysts to settle without relatively long detention times (Sreter and Szell, 1998).

III. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - Much of the results depends on the method of determining inactivation. Staining, in vitro excystation, and mouse infectivity methods provide a wide range of differing results. Ultraviolet irradiation was originally thought to be ineffective for inactivating oocysts. However, this was because excystation or dye exclusion/staining assays for estimating viability were found to not correlate with animal infectivity. Subsequent testing of UV with animal and tissue culture assay systems has shown UV treatment be very effective for inactivating oocysts (Hargy et al., 2000; Shin et al., 2001). It appears that very high levels of chlorine are required over long periods of time to render oocysts noninfectious. Ozone appears promising with an average CT value of 4.5mg.min/l to obtain 99% inactivation (Rose et al., 1997).

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. – Based on a dye-permeability study, the oocysts of *Cryptosporidium parvum* are inactivated at 37°C in 8 days, at 47°C in 4 days, and at 55°C within 2

days (Kato et al., 2001a). With anaerobic digestion, some oocysts remained viable at 37°C for 10 days.

C. Summary of survival when land applied or in watershed environments - Unknown. Laboratory studies of oocyst survival in water at temperatures from -10 to 35°C indicate that oocysts retain infectivity for mice after 16 weeks storage at temperatures of 0, 5, 10, 15, and 20°C and for 24 weeks at 5, 10, 15, and 20°C (Fayer et al., 1998). In soil, oocysts are subject to destruction when exposed to freezing or to repeated freeze-thaw events (Kato et al., 2002). Also, oocysts are found to survive for extended periods in moist soil that can last for months to years (Kato et al., in press).

D. Identification of those areas where there is no current knowledge - There is no current knowledge on the survival of the oocysts in various dewatered sludges, e.g., lime stabilized or composted material. Also, there is no information about how long oocysts would survive if they were applied to land either in biosolids or as effluent for irrigation.

IV. Detection

A. Currently used methods - In water, the EPA Method 1623 and other methods generally use filtration concentration techniques from large volumes (10 to 1000 or more liters), purification procedures, e.g., sieving, density gradient centrifugation, immunomagnetic bead separation, and flourescent antibody assays with microscopic examination (Fricker and Crabb, 1998). Polymerase chain reaction (PCR) assays have also been used. The methods are very time consuming, expensive, and experience intensive. Microscopy includes unstained oocysts viewed with differential interference contrast optics. Oocysts have been stained with fluorescent dye labeled antibody or DAPI, propidium iodide, auramine acridine orange or mepacrine and have been viewed with fluorescence microscopy. Oocysts stained with acid-fast, safranin, tetrazine, carbol fuchsin and a variety of other dyes and stains have been viewed with bright field microscopy. The microscopic methods using dyes or fluorescent antibodies do not provide information on the species or infectivity of detected oocysts. PCR uses primers for a variety of genes. Infectivity assays using cell cultures have also been reported (Slifko et al., 1999). The water methods are not really applicable to the biosolids or animal manure matrices.

1. Efficiency - Highly variable depending on recovery and detection methods. Molecular methods appear to be the most sensitive and exact for identification of species and genotypes but can be blocked by environmental contaminants.

2. Reproducibility - Variable within and between methods and highly variable between laboratories (Pontius and Clancy, 1999).

B. New methods or needs for new methodology - Need good recovery and cleaning methods. Need rapid methods that can rapidly isolate and genotype individual oocysts to allow sources of contamination to be readily identified. Need a rapid and better test for viability which correlates both with infectivity and the ability of the parasites to multiply within cells.

V. What more do we need to know? Need data on the ability of the oocysts to survive in biosolids. Information is also needed on the species and genotypes occurring in municipal wastewater.

Cyclospora

I. Introduction to the pathogen

A. Summary of life history as related to transmission (For reviews see Soave and Herwaldt, 1998; Fayer, 2000) - The life cycle of *Cyclospora cayetanensis* has not been thoroughly studied . It appears similar to other coccidian parasites. In the fecal stage, the oocyst is 8-10μm in diameter, is excreted unsporulated, and is not infectious until it sporulates. After 1-2 weeks in aqueous medium at 20-30°C oocysts sporulate, contain 2 sporocysts, each with 2 sporozoites, and are infectious. After ingestion of fecal contaminated food or water oocysts release sporozoites, which penetrate epithelial cells of the duodenum or jejunum and develop asexually into clusters of 4-6 or 10-16 merozoites approximately 1-2 μm in width by 5-6μm in length. The number of asexual generations is not known and sexual stages have not been described. A wide range of laboratory animals including nonhuman primates have been experimentally exposed to oocysts of *C. cayetanensis*, but humans are the only mammals known to acquire infection and

to excrete oocysts. Although oocysts resembling *C. cayetanensis* have been reported in the feces of a duck, chickens, dogs, and nonhuman primates, there is no evidence of transmission to humans.

B. Potential pathogenic effects on humans or livestock - Clinical signs of *C. cayetanensis* infection have been reported only for humans. These include diarrhea, nausea, vomiting, and abdominal cramps beginning 1-14 days after exposure (median incubation time: 8 days) (Soave et al., 1998). Weight loss has been reported in immune competent as well as immune compromised persons (Sifuentes et al., 1995). In an outbreak involving 45 persons with diarrhea the median number of stools per day was 7 (range: 3-35 stools/day) and the median duration of diarrheal illness was 5 days (range:1-10 days) (Anon., 1997).

C. Physical nature of the resistant stage - The oocyst stage appears similar to oocysts of other genera of coccidia. Typically, oocysts of *Eimeria*, *Isospora*, *Toxoplasma*, and *Cryptosporidium* are resistant to a wide range of chemical disinfectants including chlorine and strong acids (Fayer and Reid, 1982).

D. Structure of the resistant stage - Oocysts consist of a clearly visible wall surrounding internal cytoplasm and germplasm. Oocysts are excreted in an unsporulated state consisting of a granular cytoplasm with no distinct shape or visible nuclear material (Ortega et al., 1994). The process of sporulation in other coccidia requires oxygen and is completed when fully developed sporozoites (the infective stage) are present.

E. Composition of the oocyst wall - Unknown.

F. Discussion of what is known about potential risk - *C. cayetanensis* has been found worldwide-North, Central and South America, the Caribbean, Asia, Africa, and Eastern Europe. In endemic areas such as Nepal and Peru there appears to be a greater number of clinical cases just before and during warm monsoon months, and during summer months, respectively (Soave et al., 1998). There are no known animal reservoirs and no laboratory animal models (Mark Eberhard, CDC, personnel communication).

G. Prevalence rates in human and animal populations in the US - *C. cayetanensis* does not appear to be endemic in North America. Multi-state outbreaks associated with imported raspberries were reported in 1995, 1996 and 1997, but only in Canada in 1998 (Soave et al., 1998). Fresh raspberries were the only food common to all of the 41 cluster cases involving 1012 persons in 1997. Imported raspberries form Guatemala were banned in the U.S. in 1998 (Soave et al., 1998).

H. Geographical distribution in the US if known - Virtually all outbreaks have been associated with ingestion of putatively contaminated raspberries from Guatemala (Sterling and Ortega, 1999). One outbreak among hospital personnel was thought to have resulted from contamination of a rooftop water tank. Another outbreak was associated with mesclun lettuce and still another with basil pesto pasta salad.

I. Number of stages produced by a single infection - Unknown

J. Relating known outbreaks to pathogens in wastewater or manure - Possible exposure to sewage backup in basement of immunocompetent man in Utah (Soave et al., 1998).

II. Suspected modes of transmission

A. Effluents - Unknown. No reported incidents.

B. Biosolids - Unknown. No reported incidents.

III. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - Unknown

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - Unknown. Other coccidia are rendered noninfectious by drying and composting.

C. Summary of survival when land applied or in watershed environments - Unknown

D. Identification of those areas where there is no current knowledge - Survival longevity at different temperatures and moisture levels.

IV. Detection

A. Currently used methods - Light microscopy of fresh and acid-fast stained oocysts. Fluorescence microscopy-oocysts auto-fluoresce (Garcia, 2001). PCR (Orlandi and Lampel, 2000). These methods work with oocysts in fecal smears and those from which fecal debris has been removed. However, recovery of oocysts from fresh fruits and vegetables has been difficult or impossible. Also, recovery from effluents or biosolids would prove as difficult for this pathogen as it has for *Toxoplasma* and *Cryptosporidium*.

1. Efficiency - Poor

2. Reproducibility -Poor

B. New methods or needs for new methodology - Development of techniques to recover oocysts from environmental specimens. Develop molecular tools to detect low numbers of oocysts and accurately determine species. Must have methods for assessing viability from only one or a few oocysts.

V. What more do we need to know? How commonly does this parasite enter the treatment plants of the United States? Does the parasite pose any threat to domestic animals that ultimately might graze on land where contaminated biosolids or effluents might be applied?

Toxoplasma gondii

I. Introduction to the pathogen

A. Summary of life history as related to transmission - (For review see Dubey and Beattie, 1988). *Toxoplasma gondii* is a protozoan parasite that is capable of persisting solely as an intestinal parasite of the cat or through stages found in the flesh of various animals, including humans. Humans can be infected either through the consumption of the stage passed in the feces of the cat, the oocyst, or through the consumption of the meat of an animal that has ingested this stage, e..g., by the ingestion of pork or mutton. In either event, it is believed that the cat routinely enters into the cycle of environmental perpetuation of this parasite, and that the environmentally resistant oocyst is the stage that is important in that it is at the basis, either directly or indirectly, for almost all human infections. The oocyst of *Toxoplasma gondii*, like that of *Cryptosporidium parvum*, represents the culmination of the sexual development of this parasite within the intestinal tract. The stage that typically causes disease in humans is the tachyzoite stage that undergoes significant multiplication within the cells of various organs, especially in humans who are immunocompromised; the disease produced in these individuals depends on the major sites of the growth of the organism and can include pneumonia, cerebral abscesses, and retinochoroiditis. Another way that humans can be infected is transplacentally from mothers who are infected for the first time during pregnancy. In these cases, the tachyzoites can multiply within the developing fetus and cause severe embryological deformations and deficits.

B. Potential pathogenic effects on humans or livestock - Causes neurologic flu-like symptoms, retinitis, and potentially neurologic disease in humans. Can cause severe dysfunction in fetuses if mothers infected for the first time while pregnant.

C. Physical nature of the resistant stage - There has been very little research on the biology of the oocyst stage of *T. gondii*. This is probably because of the need to infect cats in order to produce oocysts for study, because of the highly infectious nature of this stage, and because of the tough oocyst wall. Perhaps for these reasons much less is known about the chemistry and biology of this oocyst than those of other coccidial parasites. The oocyst of *T. gondii* is about 10- 12 μm in diameter, and it is capable of remaining infectious for over a year when in the shade with a mean air temperature of 19.5°C (Yilmaz and Hopkins, 1972). Oocysts are excreted unsporulated. They must sporulate outside of the cat to become infectious. This process takes 1-5 days

depending on aeration and temperature (Dubey and Beattie, 1988).Within the fully sporulated oocyst of *T. gondii* is a pair of sporocysts each consisting of a wall enclosing four of the infective stages called the sporozoites. Sporulated oocysts appear morphologically identical to those of some species of *Hammondia* and *Besnoitia* which are not known to be infectious for humans.

D. Structure of the resistant stage - The oocyst wall of *Toxoplasma gondii* is composed of 5 layers (Dubey and Beattie, 1988). The external layer is formed while the parasite is still within the enterocyte within the feline intestine and is the 5th layer to form. As discussed under *Cryptosporidium parvum*, some individuals believe that the oocyst wall of *Cryptosporidium parvum* actually represents the equivalent of the sporocyst wall. This is due mainly to the fact that these walls both have sutures that appear to open on excystation.

E. Composition of the oocyst wall - There has been little to no work on the composition of the oocyst wall of *T. gondii* but it appears similar to other coccidia and, like them, has shown resistance to harsh chemicals under laboratory conditions, including concentrated sulfuric acid.

II. Discussion of what is known about potential risk - A risk group of special concern is pregnant women. In Europe, congenital toxoplasmosis has been reported in 10-60% of neonates and is probably the most serious form of toxoplasmosis (Fayer, 1981). Infection acquired during pregnancy is transmitted to the fetus about 40% of the time (Fayer, 1981). Of those infected, 40% have clinical signs; of those with clinical signs about 40% are severe or lethal. In 1992, the estimated annual cost of neonatal toxoplasmosis in the U.S. was $5.256 billion for income loss, special education, and medical costs (Roberts et al., 1994).

A. Prevalence rates in human and animal populations in the US - In the U.S., 0-39% of human adult subpopulations were serologically positive for *Toxoplasma*, indicating prior exposure or current infection (Dubey and Beattie, 1988). Summarizing 18 serologic studies including 4871 cats, 25% were serologically positive for *Toxoplasma* (Dubey and Beattie, 1988). A serologic survey of 12,298 pigs in the U.S. indicated 24% of feeder pigs and 42% of breeder pigs were seropositive (Dubey et al., 1991).

B. Geographical distribution in the US if known - Ubiquitous and widespread.

C. Number of stages produced by a single infection - An immune naive cat can excrete $> 10^7$ unsporulated oocysts (Dubey, 1996). They must sporulate to become infectious.

D. Relating known outbreaks to pathogens in wastewater or manure - There have been no reports relating outbreaks to wastewater or manure contamination.

III. Suspected modes of transmission

A. Effluents - Unknown. No reported incidents.

B. Biosolids - Unknown. No reported incidents but majority of oocysts probably enters the solids stream of the wastewater treatment system.

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - Not known, suspected that UV treatment would inactivate these oocysts much like the oocysts of *Cryptosporidium parvum* and the cysts of *Giardia*.

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - There is nothing known about the effects of these processes. However, with the large volumes of cat feces that are currently entering municipal wastewater treatment plants, it would be important to develop some information.

C. Summary of survival when land applied or in watershed environments - So few studies have been done that conclusions are difficult. However it is apparent that two outbreaks have been attributed to oocysts in natural waters. One outbreak was in Panama where over 30 U.S. soldiers obtained water from a jungle pond and became ill (Benenson et al., 1982); another was in western Canada where 100 acute cases and 12 congenital cases resulted from contaminated

unfiltered drinking water from a local reservoir (Isaac-Renton et al., 1998). Frenkel et al. (1975) examined the survival of the oocysts in soil in Kansas and Costa Rica, and found that they could survive up to 18 months and a year, respectively.

D. Identification of those areas where there is no current knowledge - Prevalence of oocysts in surface water and wastewater. Need to have some indication as to the survival of the oocysts in various biosolids treatment processes.

V. Detection

A. Currently used methods

1. No good environmental methods; there are good methods for clinical samples.

a. No good method for the simple and rapid detection of oocysts in water or biosolids. To detect oocysts: (1) cleaning and concentration by sieving, filtering and or density gradient centrifugation to remove debris followed by direct microscopic observation using light microscopy, differential interference contrast microscopy after sporulation; (2) PCR. The current methods are cumbersome at best. Furthermore, because oocysts of *T. gondii, Hammondia heydorni, and Neospora caninum* are morphologically indistinguishable, microscopic examination of oocysts alone is insufficient for accurate identification of the species (McAllister et al., 1998; Heydorn and Mehlhorn, 2002). To determine the identity and viability of the oocysts, bioassays in mice are required (McAllister, 1999).

b. For determining infections in hosts, there are serologic tests for antibody in the host-dye test (indirect hemagglutination assay--IHA), complement fixation test, modified agglutination test, latex agglutination test, immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA). To detect circulating soluble antigen in body fluids an ELISA test is available. To detect life stages of the organism in histologic specimens, a hematoxylin and eosin (H&E) stain, fluorescent or enzyme labels attached to polyclonal or monclonal antisera may be used.

2. Efficiency - There is no information on the efficiency of the methods used to recover oocysts from water, wastewater, biosolids or soil. Existing methods are slow and cumbersome and have the added problem that working with the oocysts can be dangerous if they are allowed to become infectious.

3. Reproducibility - There is no information relative to the recovery of oocysts from environmental samples.

B. New methods or needs for new methodology - There is a need for rapid methods that are capable of ascertaining the viability of the recovered oocysts for screening water and wastewater samples.

VI. What more do we need to know? How prevalent this parasite is in wastewater and how to best recover it from environmental samples. Also, need some indication on how resistant the oocysts are to routine sludge processing methods.

Microsporidia

I. Introduction to the pathogen

A. Summary of life history as related to transmission - (For an overview, see Wittner and Weiss, 1999.) Infections follow ingestion or inhalation of spores. The primary sites of infection are epithelial cells of the gastrointestinal and respiratory tract. Spores contain a coiled polar filament that extrudes and injects the contents of the spore into a host cell where it undergoes asexual multiplication by a process called merogony. Types of merogony differ with species of microsporidia. The most commonly reported species to infect humans, *Enterocytozoon bieneusi*, develops by karyokinesis to form multiple nuclei, followed by cytokinesis in direct contact with the host cell cytoplasm, to form multiple, independent, single nucleated, spores. The next most common species are the *Encephalitozoon* (*E. cuniculi, E. hellem* and *E. intestinalis*) that develop

by binary division within a membrane bound vacuole in the host cell cytoplasm. Eventually infected host cells rupture and release spores. Those in the intestinal tract are excreted in the feces, those in the urinary tract pass out in the urine, and those in the respiratory tract are released in respiratory secretions. The primary routes of transmission are by fecal-oral and urinary-oral routes. Microsporidia are ubiquitous and found worldwide. Although several species that infect humans also infect other animals, including monkeys, dogs, pigs, rabbits, mice, foxes, parakeets and fish, there is no direct evidence of transmission in either direction, except for the case of a child who seroconverted after close contact with a litter of puppies infected with *E. cuniculi*. Waterborne transmission has been reported in France (Cotte at al., 1999) and waterborne microsporidia have been reported in the United States (Dowd et al., 1998; Fournier et al., 2000; Sparfel et al., 1997).

B. Potential pathogenic effects on humans or livestock - The most common clinical sign of infection with *E. bieneusi* and *E. intestinalis* is diarrhea. Patients with AIDS or other immunocompromised person are at greatest risk of developing chronic diarrhea, fever, loss of appetite, weight loss, and progressive wasting disease, sometimes with complications involving the gall bladder and liver. Widely disseminated disease can result from infections with other microsporidia including sinusitis, peritonitis, nephritis pneumonia and encephalitis from *Trachipleistophora* and *Encephalitozoon* species (Wittner and Weiss, 1999). *Pleistophora* species can cause myositis. *Nosema* and *Vittaforma* species can cause stromal keratitis.

C. Physical nature of the resistant stage - The spore wall is composed of an outer layer, the exospore, and an inner layer, the endospore. The exospore is electron dense and forms a coat of uniform thickness all around the spore. Its thickness and construction vary among species, sometimes a thin, dense unstratified layer about 10nm thick to a complex multilayered structure about 200 nm thick. The chemical composition of the exospore is proteinaceous; 13 amino acids and sulfhydryl proteins have been identified in this layer. The endospore layer is electron transparent, appears structureless beneath the exospore, but contains chitin (Soule et al., 1997).

D. Structure of the resistant stage - See section C above on the physical nature of the resistant stage.

E. Composition of the spore wall - See section C above on the physical nature of the resistant stage.

II. Discussion of what is known about potential risk - Those at greatest risk are immune compromised persons.

A. Prevalence rates in human and animal populations in US - Unknown.

B. Geographical distribution in the US if known - Focal. Individual cases.

C. Number of stages produced by a single infection - Unknown.

D. Relating known outbreaks to pathogens in wastewater or manure - None documented.

III. Suspected modes of transmission

A. Effluents - Unknown; no incidents reported. The very small size of the spores would suggest that they are more likely to be present in effluents than biosolids. However, we know very little about the specific density of the spores of the different species that may pose a threat in wastewater.

B. Biosolids - Unknown; no incidents reported.

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - Using a tissue culture assay, investigators reported a 3-$\log_{10}$ reduction of *E. intestinalis* spores after exposure to 2 mg/liter of chlorine for at least 16 minutes (Wolk et al., 2000).

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - Unknown.

C. Summary of survival when land applied or in watershed environments - Unknown.

D. Identification of those areas where there is no current knowledge - Survival longevity of spores at different temperatures and moisture levels and after exposure to different disinfectants.

V. Detection - Would be very hard to detect in biosolids or effluents.

A. Currently used methods - These methods are used for clinical samples (Garcia, 2001): Immunofluorescence microscopy, chitin stains (Uvitex 2B and Calcofluor white M2R), Gram positive staining, PAS positive staining, chromotrope staining, PCR (Dowd et al., 1998b).

1. Efficiency - Detection is difficult but highly experienced persons have detected spores in feces at a limit at 5 x 10^4 spores per ml using microscopy and 10^2 per ml by PCR (Didier, personal communication; Fayer, unpublished).

2. Reproducibility - Unknown.

B. New methods or needs for new methodology - Methods for cleaning spores from environmental debris so they can be observed more readily or that inhibitors to PCR can be removed.

VI. What more do we need to know? Information is needed on the relative numbers of these pathogens entering sewage treatment plants and their occurrence in the manures of domestic animals in the United States.

Balantidium coli

I. Introduction to the pathogen

A. Summary of life history as related to transmission - Cysts are excreted in the feces of infected persons and pigs (Beaver et al., 1984). Trophozoites are found in the cecal and sigmoidorectal portion of the bowel. They reproduce by binary division. They are oval, have a slightly pointed anterior end, a thin membrane covered with cilia, foamy cytoplasm, a large macronucleus and tiny micronucleus, and measure 40 to 80 by 25 to 45 μm. At some point they transform into cysts and leave the body in the feces. Transmission is by the fecal oral route from human to human. Organisms indistinguishable from *B. coli* have been found in the buffalo, guinea pig, pig, peccary, chimpanzee, orangutan, and macaque. Pigs harbor B. coli as an asymptomatic commensal in the intestine and pigs have been implicated as a major source of infection for humans. But their role is controversial. In some areas of the South Pacific where people live in intimate contact with pigs, the prevalence rates of balantidiasis is as high as 29%. An outbreak in Truk in the Caroline Islands following a typhoon was thought to result from contamination of drinking water with pig feces (Walzer et al., 1973).

B. Potential pathogenic effects on humans or livestock - Most infections in humans are either asymptomatic or characterized by intermittent diarrhea and constipation. Chronic infections are characterized by alternating episodes of diarrhea and constipation with cramps, abdominal pain, and tenesmus. *B. coli* can invade the tissues of the bowel and cause ulcers. Perforation of the intestine has resulted in peritonitis and death. Rare complications have involved the liver, vagina, ureter, and bladder (Fayer, 2001).

C. Physical nature of the resistant stage - Cysts are round to oval, surrounded by a thick wall, contain a macro- and micro-nucleus and measure about 40 to 65μm.

D. Structure of the resistant stage - Unknown.

E. Composition of the cyst wall - Unknown.

II. Discussion of what is known about potential risk - Found world wide in humans. Prevalence is known primarily from case reports and is low. Only 722 cases reported by 1960 and very few

since then. Low prevalence may be due to a lack of recognition and inappropriate diagnostic procedures.

A. Prevalence rates in human and animal populations in the US - In a survey of confined hogs in Georgia between 1977 and 1981, the prevalence of *Balantidium coli* ranged from 7% to 29% (Marti and Hale, 1986). In swine farms in Oklahoma, 55% of 28 large swine operations and 70 small operations were found to have pigs infected with *Balantidium coli* (Morris et al., 1984). On a Danish research farm, the swine herd had almost a 100% prevalence rate of *Balantidium coli* after they reached 4 weeks of age (Hindsbo et al., 2000). In Japan, all of 88 pigs brought to a slaughter house were found infected with *Balantidium coli* with a mean cyst count of 1,150 cysts per ml of feces (Nakauchi, 1990).

B. Geographical distribution in the US if known - Unknown. Probably present in almost all swine operations whether small or large.

C. Number of stages produced by a single infection - Unknown. Pigs can produce an average of 1,150 cysts per ml of feces (Nakauchi, 1990).

D. Relating known outbreaks to pathogens in wastewater or manure - The outbreak in Truk was believed to be due to swine feces being washed into a water supply (Walzer et al., 1973).

III. Suspected modes of transmission - Transmission is by the fecal-oral route and is usually associated with poor hygiene or poor water quality.

A. Effluents - Unknown; no incidents reported. Cysts are large and relatively heavy; they probably concentrate to some extent with sludges, more so than with either *Giardia*, *Cryptosporidium*, or *Entamoeba*.

B. Biosolids - Unknown; no incidents reported. In parts of the world where human infections with this parasite are common, sludges can contain large numbers of cysts. In Bahrain, *Balantidium coli* was detected at a mean concentration of 234 cysts per ml of activated sludge (Amin, 1988). In South Australia, it was found that 70% of pigs were infected with this parasite (O'Callaghan and Langston, 1990).

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - Unknown.

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - Unknown overall. An examination of sludge in France that was stored for extended periods showed that the cysts of *Balantidium coli* were still detectable after 119 days although the cysts of *Giardia* had disappeared (Baron et al., 1989). In Lodz, Poland, in compost, a few *Balantidium coli* cysts were still detectable after 21 weeks, although the viability of the cysts was not ascertained (Gadomska et al., 1976). Work in Russia with swine manure has shown that the cysts survived biological processing of the manure but were inactivated by treatment with ammonia gas or with the addition to the sludge of 12.5% ammonium hydroxide (Cherepanov et al., 1983).

C. Summary of survival when land applied or in watershed environments - Unknown. In the Congo, cysts of *Balantidium coli* were detected in almost 20% of 92 samples of river water (Muteba, 1999).

D. Identification of those areas where there is no current knowledge - Prevalence in the United States. No knowledge about how common cysts are in effluents or sludges produced by swine operations. Unclear as to the percentage of infected individuals entering the country from areas where the infection is much more common, e.g., Latin America and Southeast Asia.

V. Detection

A. Currently used methods - Microscopic identification of cysts in stool specimens. No approved method to recover cysts from effluents or biosolids. No commercially available immunofluorescent labels.

1. Efficiency - Unknown.

2. Reproducibility - Unknown.

B. New methods or needs for new methodology - Depends on studies of prevalence. Need to produce verified assays for detection in processed manures and biosolids to be able to ascertain meaningful prevalence values in various systems. Currently, there are methods for culturing the trophozoites of this parasite (Klass, 1974), but there are no good biological assays for viability.

VI. What more do we need to know? Infectivity of the swine parasite for humans (studies suggest that the organisms in swine are somehow different from those in rats and may be less infectious to people than are cysts in human feces (Rochkene, 1976). Need to have means of ascertaining viability. Need a standard method for the identification and enumeration of cysts present in various biosolids samples.

Giardia lamblia

I. Introduction to the pathogen

A. Summary of life history as related to transmission - *Giardia lamblia* (syn. *Giardia duodenalis, Giardia intestinalis)* is a protozoan parasite with a flagellate stage called the trophozoite, parasitizing the mucosal surface of the anterior small intestine. Trophozoites multiply by binary division and can produce large numbers of organisms in an infected host. Trophozoites that enter the posterior intestinal tract are induced to secrete a wall around themselves, thus becoming encysted. After the formation of the cyst wall, the trophozoite undergoes one division to form a mature cyst containing two trophozoites capable of infecting the next host. The cyst is transmitted between hosts by direct fecal-oral contact or through contaminated water or contaminated food. After ingestion, the cyst passes through the acidic milieu of the stomach and releases the two trophozoites in the small intestine where they establish new infections. *Giardia* isolates of human, sheep, cattle and dog origin could be transmitted to *Giardia*-free rodents and were found to be rather homogenous in biochemical parameters, supporting the hypothesis that zoonotic transmission of *Giardia* may occur. However, because many studies have been based entirely on morphologic features of the parasite, it remains unclear if genetically different organisms may be hidden under the umbrella of one species (Thompson et al., 2000).

B. Potential pathogenic effects on humans or livestock - Causes diarrhea and fat absorption problems.

C. Physical nature of the resistant stage - The cyst of *Giardia lamblia* is ovoid and approximately 8 to 12 μm in its longest axis. The specific gravity of the cyst is close to 1.08. Cysts are capable of remaining viable for up to 56 to 84 days when suspended in river or lake water under winter conditions (DeRegnier et al., 1989). The dry weight of 4×10^6 intact cysts of *Giardia lamblia* has been shown to be 1 mg (Jarroll et al., 1989).

D. Structure of the resistant stage - The structure of the cyst wall of *Giardia lamblia* has been examined by Erlandsen et al. (1989, 1990, 1996), Sheffield and Bjorvatn (1977), and others. The wall is about 0.3 μm thick, composed of a combination of filamentous and particulate components, and is tightly applied to the plasma membrane of the parasite. There is a lacunar system present between the contained parasite and the cyst wall that is filled with a material of low electron density. Variability observed in the morphology of the lacunar system may represent different stages of cyst maturation. The cyst wall consists of inner and outer walls. The inner cyst wall is composed of 2 membranes. One borders the peritrophic space of the parasite and appears to serve as the attachment site for the outer cyst wall. The outer wall is composed of individual thin filaments measuring 7 to 20 nm in thickness and arranged in a tightly packed meshwork.

E. Composition of the cyst wall - Purified cyst walls of *Giardia lamblia*, based on gas chromatographic and mass-spectrometric analyses, have a total dry weight of about 43% carbohydrate (Jarroll et al., 1989, Manning et al., 1992). After treatment with 1% sodium dodecyl sulfate treatment at 100°C for 2 to 5 minutes, 86% of the total carbohydrate was found to be galactosamine.

Lectin staining suggested this sugar likely exists as N-acetylgalctosamine. Other studies found 255 nmoles of amino acids present in SDS-treated walls from 10^6 cysts but only 6.8 nmoles/10^6 in proteinase treated cyst walls, suggesting that cyst wall filaments might consist of a complex of carbohydrates and peptides (Manning et al., 1992). Cyst specific antigens identified in cyst wall vesicles are formed from acidic leucine-rich protein with multiple cysteine residues (Lújan et al., 1995; Mowatt et al., 1995). Also, it has been shown that the filaments present in the cyst wall are composed of multiple filament populations with filament diameters ranging from 7 to 15 nm (Erlandsen et al., 1989; Erlandsen et al., 1990). The large caps on at the filament initiation sites has been postulated to perhaps be similar to the globular terminal enzyme granule, chitin synthase, that is found associated with chitin microfibril formation in yeasts (Erlandsen et al., 1996). This suggests that filaments in the cyst wall are composed of a linear carbohydrate core where the formation of polymers of the N-acetylgalactosamine might be formed in an end-growth manner similar to that observed with chitin (Erlandsen et al., 1996). Other biochemical evidence, however, suggests that the N-acetylgalactosamine might be a highly branched form inconsistent with a linear carbohydrate core where the terminal structures might be glycosylating a peptide core (Erlandsen et al., 1996).

II. What is known about potential risk

A. Prevalence rates in human and animal populations in the US - As of 1997, giardiasis was the most commonly diagnosed parasitic disease in public health laboratories in the US. In 1997, cases per 100,000 population ranged from 0.9 to 42.3 for 43 different states (Furness et al., 2000). The infection is common in both beef and dairy cattle. Similarly, the infection is relatively common in dogs and cats, being reported in up to 10 to 40% of stray animals. The role of domesticated and wild animals in the transmission of *Giardia* to humans is not clearly understood.

B. Geographical distribution in the US if known - Throughout the US. Found in a survey from around the United States in influents of all 11 wastewater treatment plants sampled (Sykora et al., 1991).

C. Number of stages produced by a single infection - Naturally infected calves have been found to excrete a mean of 1180 cysts per gram of feces (Olson et al., 1997). Danciger and Lopez (1975) studied cyst production in children aged 3 to 7 years. They reported excretion of up to 2.2×10^6 cysts per gram of formed stool with an overall mean of 5.8×10^5/g. In another study (Tsuchiya, 1931), two human carriers (ages not reported) had a mean daily production of 2.1×10^8 cysts with a maximum of 7.1×10^8.

D. Relating known outbreaks to pathogens in wastewater or manure - Few outbreaks of waterborne giardiasis have been linked to wastewater.

III. Suspected modes of transmission

A. Effluents - Most cysts probably enter the effluent stream. In a study at the Montreal Urban Community wastewater treatment facility (primary treatment only, 2 billion gallons per day), the primary settling removed 76% of the *Giardia* cysts in the influent (Payment et al., 2001).

B. Biosolids - No reported incidents. The *Giardia* removed by primary clarification would have to enter the biosolids stream at a wastewater treatment plant. *Giardia* cysts probably do not survive well in biosolids. However, Mort et al (1996) found that *Giardia* in composted sludge from anaerobic digesters survived as well as cysts that were simply monitored in long-term sewage sludges. It would be necessary to verify these observations with additional methods of viability testing to insure that the process used actually did not inactivate this parasite.

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment

1. A study was conducted to evaluate the removal efficiency for *Giardia* sp. cysts of a number of wastewater treatment plants in France, 5 activated sludge systems, 3 trickling filters, and 3 waste stabilization pond systems (Wiandt et al., 2000). Cysts were detected

in raw wastewater at all treatment plants. Cyst concentrations ranged from 130 to 41,270 cysts/liter. Removal by sewage treatment was found to range between 99.5 and 99.8% for activated sludge, 99.9 and 100% for waste stabilization ponds, and up to 98.3% for the trickling filter. Despite the high removal efficiencies the range of cysts detected in final effluents ranged from < 1 to 66 cysts/liter, however, it is not known whether such cysts were viable.

2. Quantities of chlorine required to kill *Giardia* have been set for the surface water treatment rule (Clark and Regli, 1993.) Cysts of *G. lamblia* were exposed to free chlorine in buffered water at pH 5, 7, and 9 at 15°C (Rubin et al., 1989). The contact times required to obtain a 2-log reduction in cyst survival (i.e., a 99% kill) were interpolated from survival curves generated at fixed concentrations of chlorine in the range of 0.25 to about 16 mg/liter. Concentration-time (Cx t) products for 99% inactivation ranged from about 120 to nearly 1,500 mg min/liter. For *Giardia muris* cysts, concentration-time products for free chlorine obtained at 25°C ranged from a low of 50 mg min/liter at pH 5 to a high of 218 mg min/liter at pH 9 and were as high as 1,000 mg min/liter at 5 °C. It appears that *G. muris* cysts are somewhat more resistant to inactivation than *G. lamblia* cysts and rank among the microorganisms that are most resistant to inactivation by free chlorine (Leahy et al., 1987).

3. *Giardia lamblia* cysts were inactivated in water with ozone at pH 7.0 and 5 and 25°C (Wickramanayake, 1984). The concentration-time products for 99% inactivation were 0.53 and 0.17 mg min/liter at 5 and 25 °C, respectively. These products were significantly lower than those reported for chlorine.

4. UV has recently been shown to be highly efficacious in the killing of *Giardia* cysts in water and wastewater (Belosevic et al., 2001).

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - Aerobic digestion has been shown to cause a 1.4 $\log_{10}$ reduction in cysts and anaerobic digestion was shown to cause no significant reduction in cyst numbers (Chauret et al., 1999). Soares et al (1994) also found that the numbers of cysts present in anaerobically digested sludge were not affected by the treatment. However, this work did not monitor the viability of the recovered cysts, only the number of cysts present. On the other hand, Gavaghan et al. (1993) found that anaerobic sludge digestion would inactivate 99.9% of added cysts within 18 hours after they were added to the test system. Hu et al. (1996) found that *Giardia* cysts posed the highest potential risk of infection in treated sludge with numbers of cysts equal to 900/g wet weight of anaerobically treated sludge. However, there was not a good means of ascertaining what proportion of the cysts were infectious. These same authors felt that cysts would remain in stored sludge for up to a year and be present following composting.

C. Summary of survival when land applied or in watershed environments - Hu et al. (1996) applied sludges containing *Giardia* cysts to sandy soil, and they found that the cysts appeared to be destroyed within 12 weeks after soil amendment. The background prevalence of *Giardia* in surface water supplies is quite high; LeChevallier et al (1991) found *Giardia* cysts in 81% of raw water samples from 66 surface water treatment plants from 14 US states and 1 Canadian province. However, the assay did not assess the viability of the oocysts present in these waters.

D. Identification of those areas where there is no current knowledge - Additional data on survival in the environment, including the physical conditions, longevity, and any species or genotype variation, are needed.

V. Detection

A. Currently used methods - EPA Method 1623 designed for drinking water utilizes purification with magnetic beads. Oocysts are identified by IFA and assessed for viability by dye staining (Connell et al., 2000). The procedure is designed for clean water and works less efficiently with increasing solids, so no good data exists on different effluents. Also, the process is very expensive due to reagents. Available methods are genus-specific and do not give information on infectivity. There is not an approved method for biosolids.

1. Efficiency - Difficult to judge, hard to get routine laboratory data on *Giardia* cyst recovery. EPA has collected such data (Connell et al., 2000; Hsu and Huang, 2000).

2. Reproducibility - False positive and false negative rates were determined for an EPA information collection rule (Connell et al., 2000). Work should continue on the development of faster techniques that can be easily applied to biosolids samples.

B. New methods or needs for new methodology - Need better ways to isolate organisms quantitatively from biosolids and large quantities of effluents for the purpose of mass balance studies and risk assessment. Molecular or antibody techniques for species/genotype identification are to show the relationship of cysts in effluents or biosolids to those in the environments in which they are used or land applied. A method for determining the infectivity of cysts is sorely needed.

VI. What more do we need to know? Molecular data linking genotypes to cross transmission potential. A method of determining the risk posed by different species with respect to human infection is needed. Also, there is a need to know how much of the disease is linked to the lack of prior exposure to the organism as regular infection may actually reduce the symptoms associated with infections in adults. Thus, the disease may be more important in people in the developed world who routinely have clean water.

Entamoeba histolytica

I. Introduction to the pathogen

A. Summary of life history as related to transmission - The trophozoite stage (feeding body) of *Entamoeba histolytica* infects the cecum and colon (Beaver at al., 1984). Trophozoites move over the mucosal epithelium by means of extended pseudopods that attach to the surface and pull the amoebae to a site of attachment, and later to another site. To feed, trophozoites either engulf food particles or secrete proteases that aid in the extracellular digestion of materials until they are reduced to a size that can be engulfed. Trophozoites of *E. histolytica*, unlike the many nonpathogenic amoebae found in the human intestine, readily ingest red blood cells. Trophozoites multiply by binary fission and in sites where many accumulate the mucosa becomes eroded causing bloody diarrhea (dysentery). Following mucosal damage, trophozoites can spread via the blood stream to other tissues where they are capable of surviving and multiplying. The most common site of spread is the liver where they often establish "sterile" abscesses (abscesses that do not contain bacteria). Less frequently, trophozoites cause lesions in the lungs or brain. Under conditions that are not understood, trophozoites of *E. histolytica* will form cysts before they are excreted in the feces. A cyst of *E. histolytica* forms when a single amoeba secretes material that encloses it in a cyst wall. After the cyst wall is formed, the nuclear material of the cyst divides until it contains four nuclei; the cyst with four nuclei is called a mature cyst. Each mature cyst ingested by the next host will actually generate 8 trophozoites due to rapid division at the time of excystation (Dobell, 1928). Cysts typically are not excreted by individuals undergoing bouts of diarrhea or dysentery, but are found most typically in persons with formed feces, i.e., carriers. Approximately 90% of those excreting cysts are actually excreting cysts of *Entamoeba dispar,* a nonpathogenic species morphologically indistinguishable from *E. histolytica*. Monoclonal antibodies can distinguish these two species in fecal material (Diamond and Clark, 1993).

B. Potential pathogenic effects on humans or livestock - *E. histolytica* can cause severe dysentery and development of extra-intestinal abscesses can be life threatening. *E. histolytica* does not infect livestock.

C. Physical nature of the resistant stage - The cyst of *E. histolytica* is spheroid, approximately 10 to 20 μm in diameter. The specific gravity of the cyst is around 1.043 (Mirelman and Avron, 1988). Cysts can remain viable for at least 37 days at room temperature, but usually die in a few days at 37°C (Dobell, 1928). At temperatures around 4°C, cysts in water can remain viable for much longer periods (Feachem et al., 1983). The greater the numbers of bacteria in the water or the higher the temperatures, the shorter the survival times of cysts (Neal, 1974). In damp soil, cysts can remain viable for at least 8 days (Beaver and Deschamps, 1949). Cysts are quickly killed by

drying and have a thermal death point of 50°C. Cysts will resist a 1:2,500 solution of bichloride of mercury and 5% formalin for 30 minutes. Cysts in suspension are killed within 30 minutes with 1% solution of phenol, and in 15 minutes at 30°C in acetic acid. Cysts are relatively resistant to chlorination, but can be killed by hyperchlorination or iodination. For chlorination to be successful, the free chlorine has to reach a concentration of 3 ppm.

D. Structure of the resistant stage - The structure of the cyst wall of *E. histolytica* has been examined by Chavez et al. (1978) and Miller and Deas (1971). The cyst wall is about 0.7 μm thick, is composed of a numerous filaments, and contains chitin (Arroyo-Begovich et al., 1980).

E. Composition of the eggshell, cyst, oocyst, or spore cyst wall - There have not been any in-depth analyses of the chemical composition of the cyst wall of *E. histolytica*. It appears to contain a number of glycoproteins and chitin-formed fibrils.

II. Discussion of what is known about potential risk - Worldwide, it has been estimated to infect a half-billion people annually with disease in 10-15% of those infected and a resulting 50,000-100,000 deaths worldwide.

A. Prevalence rates in human and animal populations in the US - Of 216,275 stool specimens analyzed in 1987 by state laboratories, 0.9% were found to contain cysts of *Entamoeba histolytica* or *Entamoeba dispar* (Kappus et al., 1994). Despite documentation of naturally infected nonhuman primates, and experimental infection of dogs and cats, human infection from any animal source has not been documented. In 740 children from five regions of Morocco using raw wastewater for agriculture, *Entamoeba histolytica* was found in 34.3% of the children (Habbari et al., 2000).

B. Geographical distribution in the US if known - Unknown.

C. Number of stages produced by a single infection - Unknown, but estimated that an asymptomatic human (probably infected with *Entamoeba dispar*) can produce 1.5×10^7 cysts per day (Feachem et al., 1983).

D. Relating known outbreaks to pathogens in wastewater or manure - Unknown in the US. An outbreak of amebiasis and giardiasis in Sweden was traced to the intrusion of sewage into a drinking water reservoir through a spillway overflow connected to the sewer. Of 3,600 affected people, *Giardia lamblia* was isolated from 1,480 and *Entamoeba histolytica* from 106; at least 50 people were infected with both organisms (Andersson and de Jong, 1989).

III. Suspected modes of transmission - Transmission from human to human by the fecal-oral route is usually associated with poor hygiene or poor water quality. Cysts can be ingested via soiled hands of food handlers, family members, medical/hospital personnel, and other close personal contacts; food contaminated by flies; and by water contaminated with sewage.

A. Effluents - Has been reported in various effluents, e.g., in Morocco (Habbari et al., 2000). Most outbreaks have been associated with the gross contamination of drinking water with sewage through cross-flow or cross-contamination via plumbing.

B. Biosolids - Unknown; no reported incidents. Percentage that enters the solids stream as part of wastewater treatment is not known. It is known, however, that cysts are present in sewage sludges (Delmas et al., 1989).

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - Suspected that UV will cause inactivation much as for *Giardia* and *Cryptosporidium*. Chlorination will destroy cysts, but more slowly than fecal bacteria (Feachem et al., 1983). Thus, cysts may persist in waters that are judged bacteriologically safe. Chlorination has a greater inactivation effect when chlorine is free at a concentration of at least 3 milligrams per liter, at lower pH, at warmer temperatures, and with longer contact times (Chang and Fair, 1941).

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - Well run waste stabilization ponds with sufficient

cells and retention times will produce effluents free of cysts (Arceivala et al., 1970, Wachs, 1961). Most mesophilic sludge treatment processes will destroy the cysts (Cram, 1943; Kawata et al., 1977). Aerobic composting will also eliminate the cysts of this pathogen (Scott, 1952).

C. Summary of survival when land applied or in watershed environments - Basically unknown. Beaver and Deschamps (1949) showed that cysts may remain viable in damp soil for at least 8 days, in other moist cool situations for over 12 days, in water from 9 to 30 days, and at 4°C for about 3 months. Feachem et al. (1983) present a summary graph showing the survival of cysts in water at various temperatures.

D. Identification of those areas where there is no current knowledge - No real current knowledge on the survival of this organism in soil or biosolids. Also, no information on what percentage of cysts present in sewage effluents are *E. Histolytica* or *E. dispar*, which would be important for an understanding of the epidemiology of these parasites.

V. Detection - There is current acceptance of 2 species that are morphologically identical but biologically distinct. In sewage sludge there is also the potential for confusion with the potentially zoonotic porcine species *Entamoeba polecki* and with the free-living *Entamoeba moshkovskii* which is found in polluted water and sewage treatment systems (Garcia, 2001).

A. Currently used methods - There are no routinely used or standard methods for detecting this pathogen in effluents or biosolids. In humans or other animals, one uses the microscopic identification of trophozoites or cysts in stool specimens or trophozoites in tissues. Periodic Acid Schiff (PAS) staining helps to locate intensely stained trophozoites. Iron hematoxylin or hematoxylin-eosin staining is needed to identify nuclear features. Only in sophisticated modern laboratories are surface antigens (ELISA), isoenzyme patterns, and gene sequence data used for identification. For specific identification, riboprinting is almost a requirement due to the morphological identity of the different strains or species being very difficult (Clark and Diamond, 1997).

1. Efficiency - Good for fecal specimens. The EPA method 1623 may be capable of detecting these cysts on a regular basis, but no data has been presented on its efficiency in the detection of these parasites. Also, without modification, the monoclonal antibodies used in the assay procedure would not detect this pathogen.

2. Reproducibility - No known.

B. New methods or needs for new methodology - Simple reliable tests capable of detecting the cysts in water, wastewater, or biosolids along with methods for specifically identifying the parasites in the samples.

VI. What more do we need to know? Prevalence in humans and pigs in the U.S. Presence in wastewater.

Helminths
Trichuris trichiura (human source), *Trichuris suis* (swine source), *Trichuris vulpis* (canine source)

I. Introduction to the pathogen

A. Summary of life history as related to transmission - *Trichuris* (whipworm) is a genus of nematode parasites with adults that are parasitic in the cecum and large intestine of mammals (Beaver, 1984). *Trichuris trichiura* is found in people; *Trichuris suis* is found in swine. There are very few, if any, morphological differences between the eggs of these two species, and for the purpose of this presentation, they will be handled as a single unit. Within the large intestine of the host, the adults live with their anterior end embedded within the mucosa of the host and with the posterior end of the worm free within the intestinal lumen. The adult female produces eggs at the rate of somewhere around 1,000 eggs per day. The eggs are shed in the feces in the unembryonated stage and require about three weeks to become infective after being introduced to the appropriate soil environment. The infective stage within the eggshell

is a first-stage larva. People become infected by the ingestion of these eggs. The larva hatches in the small intestine, and penetrates the mucosa either at the base of the small intestine or in the anterior portion of the large intestine. Once the worms have fully developed, they can no longer move about within the intestinal tract. It takes about 3 months for the worms to reach the adult stage.

B. Potential pathogenic effects on humans or livestock - Causes infection of the cecum and colon. Only *Trichuris trichiura* causes regular infections in people. The others are rarely reported infections of people. All are potential causes of signs and symptoms of large bowel diarrhea.

C. Physical nature of the resistant stage - The eggs of *Trichuris trichiura* are characteristically barrel-shaped or lemon-shaped, and in addition to the vitelline membrane, have three layers to the shell with the outermost being brown (Garcia, 2001). The eggs also have bipolar plugs on both ends of the eggshell. When passed in the feces, the eggs contain a single celled oocyte. When passed in the feces, the eggs are brown in color, but the mucoid plugs remain clear. The eggs have a specific gravity between 1.1283 to 1.1310, the same as the eggs of *Ascaris suum* (David and Lindquist, 1982). "The eggs are much less resistant to desiccation and heat than are those of *Ascaris*, will not usually develop to the infective stage on hard clay, ashes, or cinders, and will not survive direct sun, intense cold, a putrefying medium, or action of many chemical agents. Even in a moist atmosphere, a moderately dry film of feces will support survival only a few days or weeks." (Beaver et al., 1984). It has been shown that the eggs of *Trichuris suis* are capable of successfully overwintering in Lithuania with temperatures ranging from 0°C to 20°C (Medzevicius, 1974). The eggs did not dissolve in 0.1 N HCl or NaOH, 0.5 M Na_2S, formamide, 6 M urea, 2 M $CaCl_2$, or saturated lithium thiocyanate. There was a slight dissolution of the shell and plug of the eggs in concentrated sulfuric acid. Sodium hypochlorite, 10%, caused complete dissolution of the eggshell and plug (Wharton and Jenkins, 1978).

D. Structure of the resistant stage - The structure of the eggshell has been examined by Wharton and Jenkins (1978) and by Preston and Jenkins (1984 and 1985). The shell is composed of three layers, the outer vitelline layer, the middle chitinous layer, and the internal lipid layer. The thin and dense vitelline layer envelopes the entire egg, including the polar plugs. The chitinous layer is composed of fibrils that had a diameter of 100 Å with an electron lucent core of about one-fourth the total fibril diameter. It was assumed that this represented a chitin microfibril surrounded by a protein coat. The structure of the fibrils in the chitinous layer was thought to represent helicoidal architecture, similar to the organization of chitin in arthropod cubicles. The lipid layer at the middle of the egg is about the same thickness as the chitinous layer and appears composed of parallel fibrils. The polar plugs consist of electron dense and electron lucent material with the electron-dense component appearing to consist of fibrils that are slightly larger than those of the chitin layer.

E. Composition of the eggshell - The chitinous layer of the eggshell appears to be composed of chitin fibrils that are arranged in a helicoidal architecture. The chitinous layer contains protein, and the brown color indicates that some of these proteins are stabilized by a quinone-tanning process. Wharton and Jenkins (1978) were unable to detect chitin by the chitosan test, but glucosamine in the trimethylsilyl derivative was detected in the products of acid hydrolysis of the egg shell. Infrared spectra of *Trichuris suis* egg shells appeared similar to some observed with insect eggshells. The plugs appear to contain a protein/chitin complex. Chitin that is protected by quinone tanning is believed to be protected from the action of strong acids. The greater swelling of the polar plug upon immersion in sulfuric acid, was considered to possibly be indicative of the lower proportion of quinone-tanned proteins in the plug.

II. Discussion of what is known about potential risk

A. Prevalence rates in human and animal populations in the US - Examination of 216,275 stool specimens in 1987 revealed a prevalence of *Trichuris trichiura* in the United States of 1.2% (Kappus et al., 1994). A survey of swine farms in 1984 revealed that *Trichuris suis* was present on 45% of 84 farms in 15 states (Kennedy et al, 1988). *Trichuris vulpis* in dogs was found to have a prevalence of 8.7% in Wisconsin (Coggins, 1998), and a national prevalence of 14.3% (Blagburn et al., 1996).

B. Geographical distribution in the US if known - *Trichuris trichiura* has a background prevalence based on one set of samples in the United States of 1.2% (Kappus et al., 1994). The nature of the soil transmission of this parasite would suggest that it is probably located mainly in rural areas and in larger urban populations. *Trichuris vulpis* has a high background prevalence in stray dogs (Blagburn et al., 1996), but it is probably much lower in well-cared-for dogs.

C. Number of stages produced by a single infection - Female worms produce 2,000 to 10,000 eggs per day. A typical infection can consist of a few to hundreds of worms, about one-half of which will be female.

D. Relating known outbreaks to pathogens in wastewater or manure - Humans were infected with this parasite when untreated wastewater was used for irrigation purposes in Jerusalem (Shuval et al., 1985).

III. Potential for transmission by effluents and biosolids

A. Effluents - If there is any type of primary clarification, the heavy eggs of these parasites would be expected to be present in the solid waste stream. This would also hold true in the case of treating manure containing the pig whipworm.

B. Biosolids - The eggs if present in wastewater will settle rapidly and can be expected to be found in the sludge whenever infected individuals are present in the surrounding population.

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - The eggs settle rapidly. They are not likely to be damaged by routinely used quantities of UV, ozone, or chlorination. While no studies have been reported, gamma irradiation, 200 krads, would likely inactivate the eggs of *Trichuris* as it does the eggs of *Ascaris*.

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - The treatment of eggs with routine anaerobic and aerobic digestion will have no marked effect on viability. Enigk et al (1975) found that the eggs of *Trichuris* survived thermophilic digestion for up to 5 days while those of *Ascaris* were eliminated within 3 days. The addition of caustic and ammonia will have detrimental effects on eggs at high levels at elevated temperatures of 30°C and 40°C after several days (Ghiglietti et al., 1995). When liquid pig manure was stored in concrete containers for 13 months, *Trichuris suis* eggs were found to have been 100% inactivated after 9 to 11 months while the eggs of *Ascaris suum* were still around 8% to 30% viable. Thermophilic processes — e.g. heat drying, composting, or thermophilic digestion — should inactivate these parasites in whatever matrix they may be found (Reimers et al., 1986).

C. Summary of survival when land applied or in watershed environments - When eggs were applied to a clay-flint soil in London, it was found that 21% were still infectious after 18 months (Burden et al., 1976). Using *Trichuris suis* on pasture plots in England, it was found that the eggs did not rapidly leach through the soil but remained present and were still available and infectious to grazing pigs 2.5 years after application (Burden and Hammet, 1979).

D. Identification of those areas where there is no current knowledge - Information needs to be collected on the ability of various animal manure treatment processes to inactivate *Trichuris suis*. There is a potential for minimal cross-transmission to people to occur, so it would be important to identify whether current processes are killing these eggs.

V. Detection

A. Currently used methods - The currently used method is the method published as part of 40 CFR Part 503 regulations (USEPA, 1993). The method as currently written is cumbersome and untested in its current configuration. A significant need is a thorough examination of the method and its ability to recover eggs from biosolids of different types.

1. Efficiency - Efficiency has not been established with the eggs of *Trichuris trichiura*. These eggs are smaller, at least in one dimension, than the eggs of *Ascaris*, and it

is possible that they are not recovered as well using the 400 mesh, 38 μm opening, sieves that are routinely employed to capture the eggs.

2. Reproducibility - Reproducibility has been established with the more common egg of *Ascaris*, so it is difficult to ascertain how well the work also applies to the eggs of this parasite. The heavier nature of these eggs may mean that they are not recovered as well by the 1.2 specific gravity fluids used for the flotation of the eggs away from the other solids in the sludge samples.

B. New methods or needs for new methodology - Need to have more rapid ways of isolating the parasites from large volumes of sewage sludge. Ultimately, this will probably only be achieved if various portions of the process can be mechanized so certain tasks can be repeated numerous times to increase sample size. Unfortunately, the cost recovery is probably not there to make the research and developmnet expenditure worthwhile.

VI. What more do we need to know? The potential for cross-transmission from swine to cause disease in humans is not known. Information is needed on the numbers of eggs in the sludges being produced in the United States, and on how to economically destroy the eggs in various treatment systems.

Ascaris lumbricoides (human source)/Ascaris suum (swine source)

I. Introduction to the pathogen

A. Summary of life history as related to transmission - *Ascaris* is a genus of nematode parasites with adults that are parasitic in the small intestine (Beaver et al., 1984). *Ascaris lumbricoides* is found in people; *Ascaris suum* is found in swine. There are very few, if any, morphological differences between these two species, and for the purpose of this presentation, they will be handled as a single unit. Within the small intestine, the adult worms grow to be about a foot long, and the female is highly fecund producing about 200,000 eggs each day. Her life expectancy as an adult is about one year. The eggs after passing into the environment require a period of several weeks at temperatures between 20°C to 30°C during which the single cell stage passed in the feces develops to an infective-stage larval form. The next host is infected when it ingests the infective egg. After ingestion, the larva hatches out of the egg, migrates through the intestinal wall into the liver, is carried into the lungs where it is coughed up and swallowed before reentering the small intestine. It takes about two months for the worms to develop to the point where they are ready to begin laying eggs.

B. Potential pathogenic effects on humans or livestock - *Ascaris lumbricoides* causes transitory liver and lung disease through larval migration, and adult worms a foot or so long are capable of developing within the small intestine (Beaver et al., 1984). *Ascaris suum* will typically only cause transient liver and lung disease in humans.

C. Physical nature of the resistant stage - The egg of *Ascaris lumbricoides* is broadly ovoid with a thick, transparent shell, consisting of a relatively nonpermeable innermost lipoidal vitelline membrane (not found in unfertilized eggs), a thick transparent middle layer, and an outermost coarsely mammillated albuminoid layer, usually bile-stained and tanned a golden brown (Garcia, 2001). The eggs measure around 60 μm in diameter when spherical, but can be elongated to 75 μm by 50 μm. Eggs require the presence of oxygen and favorable temperatures for embryonation, but are highly resistant to dessication and low temperatures. If eggs are maintained in anaerobic conditions, they will remain in the one-celled stage, but after air becomes available, 75% of eggs that have been stored in this manner will be capable of developing to the infective states. The rate of oxygen consumption by the developing eggs drops during the first day after the beginning of embryonation, reaches a peak consumption about 12 days after the beginning of embryonation, and by 25 days falls to near zero where it remains for up to 140 days after the initial development (Passey and Fairbairn, 1955). The specific gravity of the egg is around 1.1299 (David and Lindquist, 1982). Fairbairn (1957) reviewed the response of the eggshell to strong chemicals: acid and alkali dissolved the external coat, hypochlorite will dissolve the thick shell, but the thin inner membrane will still remain protective. This membrane can be dissolved with chloroform, ethyl ether, alcohols, phenols, and cresols. It melts at around 70°C.

This membrane is permeable to the respiratory gases and to hydrogen cyanide, ammonia, and carbon monoxide, but it is impermeable to cyanide, azide, and ammonium ions and to salts in general. Fairbairn (1957) argues that it is unclear in spite of all evidence whether the eggshell is actually permeable to water, and suggests that experiments with deuterium should be applied to examine this problem.

D. Structure of the resistant stage - Lysek et al. (1985) examined the ultrastructure of the eggs of *Ascaris lumbricoides*. They recognized a mucopolysaccharide uterine layer forming on the egg surface that was irregular and typically 1.5 μm thick. Underneath the uterine layer is the thin vitelline layer which originates from the oolemma of the fertilized oocyte. Under this layer is the thick chitin-protein layer that is about 2 μm thick. Finally, underneath this layer is the inner lipid (ascaroside) layer that reaches a thickness of about 1 μm.

E. Composition of the eggshell - Wharton (1980) reviewed the structure of nematode egg shells and along with the review of Fairbairn (1955) are the basis for the following discussion. The internal layer of the egg of *Ascaris lumbricoides* is a lipid layer consisting of 25% proteins and 75% lipid. The lipid fraction contains a mixture of □-glycosides called ascarosides. These consist of a sugar moiety (glycone), 3,6-dideoxy-L-arabinohexose (ascarylose) and a long chain secondary alcohol (aglycone). Ascarosides can be divided into three classes: the mono ascarosides, the diol ascarosides, and the diol di-ascarosides. The thick clear middle layer of the *Ascaris* egg is the chitinous layer. The presence of chitin in *Ascaris lumbricoides* eggs has been demonstrated by x-ray crystallography. Sromová and Hejtmánek (1987) used a derivative of stilbenedisulfonic acid which forms a specific bond with compounds of the hexapyranose type, e.g., chitin, to show that the chitin layer in thin sections of eggs fluoresced as would be expected of material containing chitin. The chitin is soluble in hot, concentrated mineral acids, in sodium hypochlorite and antiformin, and also in hot alkali. The basic substrates for chitin formation are glucose, ammonia, and acetate. All of these are present in the oocyte: glucose in the form of glycogen and trehalose, ammonia in amino acids and proteins, and acetate in ascaroside esters and probably also as triglycerides. On the outside of the chitin layer is a layer applied by the uterus that hardens to form the characteristic sculptured appearance. When the eggs are first removed from the uterus, they are essentially colorless, whereas, those recovered from feces are usually golden-brown colored. The color development has been attributed to the staining of the outer coat by bile pigments, but it has also been suggested that the outer layer undergoes a tanning process through the process of quinone-tanning of the proteins in this layer. In eggs that are freshly removed from the uterus of the worm, the outer layer can be digested with pepsin or papain and dissolved readily in 0.1 N mineral acids or alkalis. Once passed in the feces of the host, the outer layer is no longer digested by pepsin or papain, and it is no longer soluble in dilute acid or alkali. It continues to be possible to dissolve the membrane with hypochlorite solutions.

II. Discussion of what is known about potential risk

A. Prevalence rates in human and animal populations in the US - Worldwide, about 1 billion people are believed to be infected or to have been infected with this soil-transmitted infection. Kappus et al. (1994) reported that *Ascaris lumbricoides* was still present in 0.8% of 216,275 stool samples examined by state diagnostic laboratories in 1987. Kennedy et al. (1988) reported that *Ascaris suum* was present on 70% of 84 swine farms from 15 states.

B. Geographical distribution in the US if known - This is not known in people, however, it is probably highest in rural areas where transmission occurs, in urban centers where there are a lot of immigrants, and perhaps within poorer communities within urban centers where transmission is possible. Within swine, it seems to be persistent even in large factory farms, and with the greater demands for external access for confined animals, the prevalence is likely to increase. Viable *Ascaris* eggs were found in 27 of 27 municipal wastewater treatment plants in 5 southern states in the US as reported in a survey of Mississippi, Louisiana, Texas, Alabama, and Florida; the average number of eggs per plant was 9,600 per kg dry weight of sample (Reimers et al., 1981). In the four northern states of New York, Ohio, Minnesota, and Washington, it was reported that viable eggs were recovered from 48% of sludges with about 710 eggs per kg of dry weight of sludge (Reimers et al., 1986).

C. Number of stages produced by a single infection - A single *Ascaris* female is capable of producing up to 200,000 eggs per day for a period of about 6 months. Infected children will produce an average of 3,000 eggs per gram of feces, or somewhere around 1 million eggs or more per day during an infection (Mello, 1974). The number of eggs produced by infected pigs is similar (Nilsson, 1982).

D. Relating known outbreaks to pathogens in wastewater or manure - None reported.

III. Potential for transmission by effluents and biosolids

A. Effluents - No reported incidents. If there is primary settling, the eggs will typically not be found in effluents of plants operating efficiently.

B. Biosolids - No reported incidents. The effect of most treatment plants is to concentrate the eggs with the biosolids.

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - The eggs settle very well within sewage treatment plants. There is no reason to believe that the typical quantities of any of these products as routinely used for the disinfection of water would have any effect on the eggs that might be present.

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - The eggs of *Ascaris* have been chosen by the US EPA as an indicator organism because they are relatively resistant to most processes and considered representative of what effects different processes may have on helminth egg viability.

1. Thermophilic anaerobic digestion is considered by many to be the most effective stabilization method for the reduction of viable *Ascaris* eggs. Mesophilic anaerobic digestion (35°C) reduced egg viability by 30% to 50%, while digestion at 49°C reduced viability by 90% (Pike and Davis, 1984). More recent work has also shown that there can be a marked reduction at 37°C, but that some eggs remain viable in digesters with a 10 day detention time (Kato et al., 2001a). Similarly, eggs held at 47°C underwent significant inactivation within 2 days, and were very rapidly destroyed at 55°C.

2. Aerobically digested biosolids at elevated temperature have been shown to cause 100% inactivation of eggs by 4 days (Reimers et al., 1981); however, more recent work has shown that eggs remained viable in digesters at 47°C for 8 days with a significant portion still being viable at 10 days (Kato et al., 2001a).

3. Alkaline stabilization has the advantage that high pH will often release ammonia gas which is highly toxic to helminth eggs. When an ammonia concentration of 0.1% was introduced at 52°C, a one day detention time resulted in 100% inactivation, whereas at 25°C only 28% inactivation was accomplished after 10 days (Reimers et al., 1996).

4. Composting, if performed adequately with proper temperature control, will inactivate *Ascaris* eggs as long as the process is properly mixed.

5. Lagoon storage in waste stabilization ponds has been shown by Nelson et al. (2001) to have some effect on *Ascaris* eggs, but viable eggs were found in sediments that were considered on the basis of sludge cores to be over 10 years old.

6. Gamma irradiation at 200 krads will totally inactivate *Ascaris* eggs (Reimers et al., 1986; Kato et al., 2001b).

C. Summary of survival when land applied or in watershed environments - The T-90 die-off times were examined by Little et al., (1991) on sludges land applied in Texas, Ohio, and Louisiana. In biosolids that were land applied and tilled into the soil, the number of months before 90% of the eggs had died was around 36 (3 years). When the sludge was not tilled into the soil, it took only about 2 to 10 months before 90% of the eggs were inactivated. Mizgajska (1993) showed

that eggs applied to soils tended to stay in the first 10 cm, and no matter what soil type was examined there were viable eggs present in samples of all soils taken 17 months after application.

D. Identification of those areas where there is no current knowledge - Mainly need to generate better die-off curves for different treatment processes.

V. Detection

A. Currently used methods - The currently used method is the method published as part of 40 CFR Part 503 regulations (USEPA, 1993). The method as currently written is cumbersome and untested in its current configuration.

1. Efficiency - Efficiency has been found to be good using methods similar to the 503 method. Little et al. (in press) have found that there was a 60% or greater accuracy and a percent variation from the mean density of between 3 and 30%. However, this method used only about 30 grams of total solids, and the new 503 method uses some 300 grams of total solids. Thus, it would be expected that the recovery rates could go up, but the reality of performing the method would suggest that they will be markedly lower.

2. Reproducibility - As noted above, the reproducibility of the method utilized by Little et al (in press) is relatively good. There is currently no good information on the reproducibility of the 503 method.

3. Viability - Viability is determined by embryonation. This works well for anaerobic systems, but in aerobic systems or soil, many eggs will have embryonated before the assay is run, thus, it becomes necessary to look for movement within the eggshell to confirm a viable larva. This can be very tedious.

B. New methods or needs for new methodology - A simplified method should be developed and verified so that it can be run by different laboratories easily and inexpensively. Importantly, there is also a need to develop a more rapid method of assessing viability than waiting for the embryonation of cultures to the infective stage.

VI. What more do we need to know? There is a need to determine the current prevalence of these eggs in domestic biosolids and to have a method that can be quickly run by diagnostic laboratories around the United States.

Taeniid Tapeworm Eggs

I. Introduction to the pathogen

A. Summary of life history as related to transmission - The life histories of all taeniid tapeworms require a carnivore final host wherein the adult tapeworms live within the small intestine (Beaver et al., 1984). For *Taenia solium* and *Taenia saginata*, the final host is a human being or a pig and the intermediate hosts are either a pig or a cow, respectively. For *Echinococcus granulosus*, the final host is a dog and the intermediate host is a ruminant. In all cases, the intermediate host becomes infected by ingesting the infective egg that is passed in the feces of the final host. For *Taenia solium* (called the pork tapeworm because humans are infected by eating pork), the eggs hatch in the small intestine of the pig, and the contained larva, the oncosphere, migrates into the intestinal mucosa into mesenteric venules and are then carried throughout the body. The larva typically establishes in muscles where each develops over the next two months into a single infective form that is called a cysticercus. The final host becomes infected by the ingestion of meat (measly pork), that contains the cysticercus stage of the parasite. The life cycle of *Taenia saginata* (called the beef tapeworm because humans are infected by eating beef) is similar, with the difference being that the intermediate host is a cow, and human beings become infected by the ingestion of measly beef. The adults of *Taenia solium* and *Taenia saginata* are quite large reaching lengths of several meters within the intestine of the human being. In the case of *Echinococcus granulosus*, the small adults which are only millimeters long, live embedded in the mucosa of the small intestine of their canine hosts. The eggs are shed in the feces and are ingested by grazing ruminants. In the ruminant intermediate hosts, the larvae typically develop

in the liver after hatching in the small intestine where they develop into a large, several cm in diameter or larger, larval stage called a hydatid cyst each containing hundreds to thousands of potentially infective larvae called protoscolices. Dogs become infected when they eat the viscera of infected ruminants. The adults of these tapeworms typically cause few if any signs of infection in the final host. The great danger of these tapeworms is the ability of the larvae of *Taenia solium* and *Echinococcus granulosus* to utilize human beings as potential intermediate hosts. Thus, the cysticerci of *Taenia solium* and the hydatid cysts of *Echinococcus granulosus* are capable of developing in people who have ingested the egg of these parasites. These larvae cause space-filling lesions due to their size, and depending on their location can cause severe disease in people that are infected with the larvae.

B. Potential pathogenic effects on humans or livestock - The adults cause little effect in humans. The larvae of *Taenia solium* can be serious pathogens in people if they ingest the eggs. The eggs of *Taenia saginata* are infectious to cattle. The eggs of the canine species of *Taenia* do not typically infect people, but those of *Echinococcus* can cause significant human morbidity through the hydatid stage. Fortunately, canine infections with *Echinococcus* spp. are rare in the United States.

C. Physical nature of the resistant stage - The eggs of these tapeworms are not morphologically distinguishable (Garcia, 2001). Thus, when the eggs are found in human fecal samples, it cannot be determined without further effort whether the human is infected with the pork or the beef tapeworm. Similarly, if eggs are found in soil, pasture, or sludge samples, it cannot be determined if the egg is a species of *Taenia* or *Echinococcus* without additional effort. The eggs of these tapeworms are spherical and measure 31 μm to 43 μm in diameter, and they are typically golden-brown in color. The eggs are fully mature and infective when they leave the host. The infective egg contains a fully formed larva, the oncosphere which is distinguished by its possession of six small hooklets that are utilized for locomotion and as aids in the penetration of the intestinal mucosa of the intermediate host. The eggs of taeniid tapeworms have a specific gravity of around 1.2251 (David and Lindquist, 1982).

1. It has been shown (Froyd, 1962) that the eggs of *Taenia saginata* are capable of remaining viable for up to 168 days at 4°C to 5°C when stored in a solution of weak (1:10,000) thimerosal. Veit et al. (1995) have shown that common disinfectants utilized as specified for other purposes in Europe had little effect on the infectivity for the eggs of *Echinococcus*. The disinfectants used included phenols, aldehydes, phosphoric acid, ethanol, and trichloroacetaldehyde. They also found that these eggs could remain viable in tap water for up to 240 days at -18°C, and up to more than a year at 4°C. When eggs were at 4°C and a relative humidity of 85% to 95%, they remained infectious for at least 75 days but less than 111 days. However, at 45°C, the eggs survived at this relative humidity for only 1 to 2 hours. Similarly, in water at 43°C, eggs did not remain infectious after 4 hours at this temperature. At 25°C and at 27% relative humidity, eggs remained infectious for 24 hours but not for 48 hours. At 43°C and at 15% relative humidity, the eggs remained infectious for 1 hour but not 2 hours. By placing eggs in proglottids in nylon bags on soil, these same workers found that the eggs survived up to 240 days in conditions where the temperature extremes were from -15°C to 27°C. However, in other experiments, the survival times were much shorter. When mixed with soil, Jepson and Roth (1949) found that eggs persisted up to 159 days during the winter in Denmark. Eggs required 40 krads of gamma irradiation to prevent the development of infective larvae in intermediate hosts (Veit et al., 1995).

2. The embryophore of the eggs of taeniid tapeworms will rapidly disassemble in the presence of sodium hypochlorite (Crewe and Owen, 1979). The sodium hypochlorite causes the keratin blocks composing the eggshell to rapidly come apart. Ultimately, if the concentration of hypochlorite is sufficient, the contained embryo will also be destroyed.

3. It is unclear which portion of the protective barrier actually serves to protect the contained embryo. There appears to be some protection offered by the external blocks (see D. Structure of the resistant stage, below), but permeability to various compounds seems to be controlled by the internal oncospheral membrane. Of course,

the contained larva also has its own surface which would afford some modicum of protection.

D. Structure of the resistant stage - The protective barrier for the oncosphere larval tapeworm that develops, and which is exposed to the environment, is a layer of thick prismatic blocks that appear to be held together by some type of glue-like material (Wang et al., 1981). The blocks appear to be about 5 μm long and 1 μm in diameter causing the shell-like embryophore to be about 5 μm thick around the entire embryo. The blocks are thickest on the external surface of the embryophore and become increasingly narrower as they approach the embryo. Internally, the prismatic blocks are embedded in a granular layer. Underneath the block and granular layers of the embryophore is the oncospheral membrane.

E. Composition of the embryophore wall - The blocks composing the embryophore have been shown to be composed of keratin by histochemistry, chromatography, and infrared spectroscopy (Johri, 1957; Morseth, 1965). The composition of the oncospheral membrane is not known, but it is thought to very likely be a lipoprotein. It is known that bile salts can apparently change its permeability relative to certain vital dyes such that after bile treatment the dyes are capable of staining the contained embryo.

II. Discussion of what is known about potential risk

A. Prevalence rates in human and animal populations in the US - Infections with *Taenia solium* and *Taenia saginata* are low in the United States, however, infections in countries from which we have immigrant populations can be very high. Parts of Latin America from which immigrants may enter the United States can have prevalence rates as high as 1% to 5% (Borda et al., 1996; Schantz et al., 1992). Dogs are the sole source of *Echinococcus* eggs and the typical dog that would be infected is a rural animal that would not introduce eggs into the wastewater treatment system except through storm runoff. Fortunately, infection of dogs with this parasite is very rare in the United States.

B. Geographical distribution in the US if known - Sporadic. Accompanies migrant workers and thus is found rurally and in large cities to which they immigrate.

C. Number of stages produced by a single infection - Tapeworms can be meters long and shed segments for months with each segment containing more than 1,000 eggs.

D. Relating known outbreaks to pathogens in wastewater or manure - There have been cases of animals infected when grazing pasture was irrigated with untreated effluent (Hsoe et al., 1990).

III. Potential for transmission by effluents and biosolids

A. Effluents - No reported incidents. Eggs are very heavy and will concentrate with the solids.

B. Biosolids - No reported incidents. Taeniid tapeworm eggs are amongst the heaviest of eggs, so it is to be expected that they will rapidly concentrate with the solids portion of the wastewater stream.

IV. Effects of biosolids processing

A. Summary of effects of settling, UV, ozone, and chlorine treatment - These processes are likely to have very little effect on the viability of these eggs.

B. Summary of effects of biosolids processing, anaerobic digestion, aerobic digestion, lime stabilization, composting, drying, etc. - Some believe that mesophilic anaerobic digestion will inactivate the parasite, but others have suggested that this will not occur at mesophilic temperatures (Feachem et al., 1983).

C. Summary of survival when land applied or in watershed environments - Feachem et al. (1983) provide a summary table showing that eggs applied to grass or soil are cable of surviving anywhere from several days to almost 1 year depending on the temperature.

D. Identification of those areas where there is no current knowledge - Really have no good current knowledge on inactivation by routine sewage treatment. Composting would be expected to destroy the eggs, but other routinely used processes that are not thermophilic have not been examined in any detail.

V. Detection

A. Currently used methods - The currently used method is the method published as part of 40 CFR Part 503 regulations (USEPA, 1993). The method as currently written is cumbersome and untested in its current configuration.

1. Efficiency - There are no good data for egg recovery. The eggs are heavy, and there is a good chance that the specific gravity of the flotation solution, sp.g. = 1.2, will not bring the majority of the eggs to the surface. This has never been carefully examined.

2. Reproducibility - Unknown.

3. Viability - There is no good test for viability, although some vital staining methods seem helpful (Wang et al., 1997).

B. New methods or needs for new methodology - As with most of the recovery processes, there is a need to develop methods where the initial steps can be automated to allow larger numbers of samples to be processed. The vast majority of time is spent in sample processing with inherent potential problems with technique by operators. Although the final microscopic observations are also tedious, they actually require less time than the processing of each sample.

VI. What more do we need to know? There is a need to know where the eggs are within the biosolids of the United States. There is a need for a better method for egg recovery that has been verified.

Summary

The parasites can basically be classified into four groups. The Apicomplexan protozoa (*Cryptosporidium, Cyclospora, Toxoplasma*) are probably to some extent all similar relative to the effects of different disinfection while they probably settle at markedly different rates. The Microsporidia are currently an unknown commodity in that they have been little studied relative to wastewater treatment. The "soft-shelled" protozoa, (*Balantidium*, *Entamoeba*, and *Giardia*), are probably similar in that they are likely to persist to some extent in effluents but are probably subject to fairly rapid destruction when in biosolids. The helminths are probably all fairly similar relative to settling with the biosolids and also are probably very similar in their resistance to disinfection with *Ascaris* and *Trichuris* being more resistant to destruction than the eggs of the taeniid tapeworms.

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Parasites–Sludge/Biosolids Workgroup: Discussion Summary, Conclusions and Recommendations

Introduction

The workgroup examined issues related to parasitic pathogens in sludge/biosolids. The workgroup generated a list of parasites of concern. Clostridium spores were discussed as a potential indicator of treatment effectiveness and participants concluded that more studies are needed before this organism can be recommended as a suitable indicator. This workgroup ranked parasites according to the hazards that they posed to human health. Concerning the development of antimicrobial resistance, they concluded that neither antiprotozoal nor anthelminthic resistance is likely to develop in biosolids. They also prioritized research needs with the highest priority being the development and validation of detection and identification methodology for parasites.

Participants in this workgroup were: Dwight Bowman, Leader; Frank Schaefer, Facilitator; Christine Bean; Joseph Farrell; Erwin Faulmann; Donald Hendrickson; Hugh Mainzer; Robert Reimers; Andrea Vicari; amd John Walker.

Parasitic Organisms

Using the list prepared by Fayer and Bowman as a starting point (Table 1 in Concerns Related to Protozoan and Helminth Parasites in Biosolids and Animal Wastes, the paper preceding this section), workgroup participants identified additional parasitic organisms that were not discussed in the keynote presentations. The workgroup then developed a list of parasites of concern that includes some species of Protozoa, Nematodes and Cestodes (tapeworms) for use in the remainder of the discussions:

- Protozoa: Amoebas; *Cryptosporidium*; *Cyclospora*; *Giardia*; and *Microsporidia* (including *Encephalitozoon* spp.; *Enterocytozoon* spp.; *Nosema* spp.; *Pleistophora* spp.; *Trachipleistophora* spp.; and *Vittaforma* spp).
- Nematodes: *Ascaris*; *Baylisascaris*; *Necator* (hookworm); *Toxocara*; and *Trichuris*.
- Cestodes (Tapeworms): *Echinococcus granulosus* and *E. multilocularis, Taenia* spp.

Indicator Organisms

Indicator organisms are used to detect the presence of pathogens and to measure treatment efficiency. Because of the low number of Ascaris eggs typically found in domestic sewage sludge in the United States, it may be preferable to identify another commonly found organism to be used as an indicator for regular screening processes. The prime organism considered by discussion participants was *Clostridium* perfringens which is found at 10^4 to 10^6 spores per gram of raw sludge. *Clostridium* assays could be done in 2 to 3 days as a screening process, while Ascaris assays currently take 4 to 6 weeks to get results which may be dependent upon the number of samples taken. For example, when 500 to 1,000 samples were tested for the presence of *Ascaris*, only 1% of the samples were positive. There was also some discussion regarding the possible effect of geographical or seasonal factors on occurrence of *Ascaris*.

A point in favor of using *Clostridium* as an indicator of treatment effectiveness is that it appears to be inactivated by penetration of non-charged species into the spore membrane, similar to inactivation of *Ascaris* ova. Arguments against using *Clostridium* include: it is ubiquitous in the environment, it is inactivated by sodium hydroxide solutions at pH 12, and it is only inactivated at temperatures of 120 to 140°C. The workgroup concluded that more study is needed to determine matrix and treatment process effects on

Table 1. Relative Ranking of Parasitic Pathogens With Respect to Hazards Posed to Human Health

Parasite	Rank[a]	Likely to Occur in Biosolids	Worker Safety	Survival in Tilled Soil (After Biosolids Application)
Ascaris	3	Yes	Little to no risk if good hygiene exhibited	Years
Toxocara	2	Yes	Little to no risk if good hygiene exhibited	Years
Baylisascaris	1	Yes	Little to no risk if good hygiene exhibited	Years
Trichuris	3	Yes	Little to no risk if good hygiene exhibited	Years
Taenia	2	Yes	Little to no risk if good hygiene exhibited	Months
Echinococcus	1	No	Little to no risk if good hygiene exhibited	Months
Cryptosporidium	1	Yes	Little to no risk if good hygiene exhibited	Months
Cyclospora	3	Yes	Little to no risk if good hygiene exhibited	Months
Toxoplasma	1	Yes	Little to no risk if good hygiene exhibited	Months
Giardia	3	Yes	Little to no risk if good hygiene exhibited	Weeks
Microsporidia	2	Yes	Little to no risk if good hygiene exhibited	Not known

[a]Relative hazard to human health based on immune status, health impact, and availability of therapeutic treatment: 1 = high hazard; 2 = medium hazard; 3 = low hazard.

Clostridium occurrence, and on correlations between *Cryptosporidium* oocysts, *Ascaris* ova and *Clostridium* spores, before a recommendation can be made on the suitability of *Clostridium* as an indicator of treatment process effectiveness.

Ability To Assess Risks

There is an effort to develop better policies with respect to risk assessment that would include cost benefit analysis. There are different approaches to risk assessment relative to a particular pathway, given pathogens and consequences. One suggestion from the workgroup was to begin with qualitative assessments and move into quantitative risk assessment. It was suggested that perhaps the relative risks should be examined more closely to try and determine what the biggest risks are from a public health perspective. While understanding that assessing risks is not an exact science, there is a need to be aware of the purpose and limitations associated with the risks. Knowledge gaps need to be identified and eliminated.

To begin an examination of the ability to assess risks to health, the workgroup suggested that one should first identify the outcomes of concern (i.e., specific human health impacts, environmental impacts, etc.). The workgroup participants developed a relative ranking for each parasitic pathogen through a consensus process. In arriving at the ranking, the discussants considered the immune status of the exposed population, the human health impact (severity of disease), and whether or not effective therapeutic treatment is available for the parasite of concern. It should be emphasized that the relative ranking is related more to infection with the organism per se rather than to the hazard associated with exposure to biosolids. Table 1 summarizes the risk scores (rank) of the parasitic pathogens with respect to hazards posed to human health along with information on their likely occurrence in biosolids, risk to worker safety, and survival in tilled soils. Transport of parasites through the soil is less of a concern than it is for bacteria and vi-

ruses since it is dependent upon size and surface characteristics. However, it was noted that transport of parasitic pathogens is a very large data gap.

Workgroup participants also addressed the issue of antimicrobial resistance. They concluded that neither antiprotozoal nor anthelminthic resistance is likely to develop in biosolids because parasites, unlike bacteria, will not grow or multiply in the biosolids matrix. Therefore, there is no growth of resistant organisms or selection for resistance in the biosolids matrix. The development of anthelminthic resistance is likely to occur only in the host animal since there are no mechanisms for worms to become resistant, no genetic exchange between parasites in digesters of a treatment plant, and no known risk from biosolids.

Detection/Analytical Capabilities

In general, the workgroup expressed concern that current detection/analytical capabilities are not meeting needs for sampling and detection of parasites in sludges and biosolids. The workgroup believes that available sampling and detection methods are inadequate, often with researchers at a loss as to how many times, where and when to sample. In other words, current detection methods are at a critical deficiency. Below is a list of factors important to detection and identification methods, with comments on the state of the science or research needs next to each:

1. Quality Assurance/Quality Control (QA/QC). The QA/QC protocol is used to measure accuracy. The workgroup believes that quality assurance questions and issues should be explored and disseminated to the public.

2. Quality of Spike. Variability was identified as a problem with the Quality of Spike method. Also, there are cost issues associated with a duplicate spike versus a three-way split spike.

3. Preservation.

4. Quenching.

5. Matrix Effects.

6. Validation and Round Robin Testing. Methods 1622 and 1623 are available, however, there is currently no validated test or round robin testing.

Processing/Control Technologies

Workgroup participants discussed concerns relating to both Class A and Class B treatment processes with regard to parasitic pathogens. Concerns associated with currently used Class A biosolids treatment processes are indicated in Table 2.

Table 2. Processing/Control Technologies for Class A Biosolids

Time/Temperature Processes	Concerns
Heat Drying	None
Composting	Uniform exposure of parasites through mixing
Thermophilic Aerobic Digestion Process to Further Reduce Pathogens (15 days @ 55°C)	Short circuiting of process
Thermophilic Anaerobic Digestion	Short circuiting of process
Batch Pasteurization or Plug Flow	None
Lime or Alkaline Pasteurization (70°C for 30 min.)	Uniform exposure of parasites through mixing
Advanced Alkaline Stabilization	Temperature, ammonia content, uniformity
Oxyozonation (Synox)	pH, nitrous acid, uniformity, limited testing
Anaerobic Digestion with Lagoon Storage and Drying Beds	Limited data on parasite reduction
Gamma Radiation (200 kiloRads)	None

The workgroup commented that Class A biosolids processes are generally not described in sufficient detail to allow adequate monitoring of process effectiveness. They suggested that Hazard Analysis and Critical Control Point (HACCP) evaluation be used to determine those parts of the processes that should be controlled. They also questioned whether biosolids produced by Alternative 3 and Alternative 4 treatment processes should be considered Class A solely upon the basis of testing for enteric viruses and helminth ova because of deficiencies in the available testing methods.

The workgroup also considered the ability of each parasitic pathogen to survive Class B processing treatments. The Class B processes that were considered are anaerobic digestion, aerobic digestion, and lime stabilization. Participants scored each parasite qualitatively with a plus sign indicating survival and a minus sign indicating inability to survive treatment, generally (Table 3). There was inadequate data to determine survivability of several parasites, and the methodology to detect the protozoa in biosolids and to determine their survivability is questionable at best.

From the survivability scores presented in Table 3, it appears that the most resistant parasites are the Nematodes and Taenia species. During the dialogue, it was also noted that the effectiveness of certain treatment processes, such as lime stabilization, appears to be affected by seasonality (specifically by temperature).

After discussing site restrictions for Class B biosolids, workgroup participants made the following recommendations and indicated that they are open to discussion and should be re-examined: 1) Above ground food crops with harvested parts touching soil mixture to which Class B biosolids have been applied shall not be harvested for 14 months to ensure safety. (Crops could be planted one year after the biosolids have been land applied.) Concern was expressed that this may not be long enough because variability of

Table 3. Survivability[a] of Parasitic Pathogens Through Class B Biosolids Treatment Processes

Organism	Class B Process		
	Anaerobic Digestion	**Aerobic Digestion**	**Lime Stabilization[b]**
Nematodes			
Ascaris, Toxocara, Baylisascaris	+	+	+
Hookworms	-	-	-
Trichuris	+	+	+
Cestodes (Tapeworms)			
Taenia spp.	-?	-?	-?
Echinococcus granulosus	-?	-?	-?
Echinococcus multilocularis	-?	-?	-?
Protozoa			
Cyclospora	?	?	?
Toxoplasma	?	?	?
Cryptosporidium	+/-?	+/-?	+/-?
Giardia	+/-?	+/-?	+/-?
Amoebas	-	-	-
Microsporidia			
Encephalitozoon			
Enterocytozoon			
Nosema	?	?	?
Pleistophora			
Trachipleistophora			
Vittaforma	?	?	?

[a]"+" = survives and "-" = does not survive the indicated treatment process. Blank cells indicate insufficient data to make a determination. "?" = may not be a fecal problem.

[b]Lime stabilization may not work as well in winter as during summer because lower temperatures result in decreased free ammonia release.

factors such as climate and humidity can affect survivability. 2) Class B biosolids should be land applied in the spring. 3) 50-month restriction.

Prioritized List of Research Needs

Discussion participants ranked research needs for parasites in sludges and biosolids as High, Medium, or Low through a consensus process. The research needs and their priorities are shown in Table 4. The highest priority was assigned to the development and validation of parasite detection methodology. An example of a medium priority research need is evaluation of other assays for *Cryptosporidium* and *Giardia* (although these are not likely to survive Class B treatments). Of lower priority are studies that will shed light on the effects of Class A or Class B treatments on embryonated versus unembryonated *Ascaris* eggs.

Table 4. Prioritization of Research Needs for Parasites in Sludges/Biosolids

Priority Ranking	Suggested Research Needs and Outstanding Questions
High	Development and validation of parasite testing methodology
Medium	*Cryptosporidium* and *Giardia* must have a viability assay other than dye permeability
Medium	Conduct a broad survey of parasites in sludge (last done in the 1980s)
Medium	Are people in proximity of application showing evidence of exposure?
Medium	Is runoff from land application a problem?
Medium	Revisit the Class B site restrictions with regard to all parasites
Medium	Determine mass balance for treatment processes
Low	Relationship between embryonating state of *Ascaris* eggs and reaction to treatment
Low	Determine infective dose for some parasites
Low	Study the mechanism of parasite inactivation

Parasites—Animal Wastes Workgroup: Discussion Summary, Conclusions and Recommendations

Introduction

This workgroup addressed known and emerging parasitic organisms in animal wastes. The participants categorized the public health significance of the organisms by using a consensus relative ranking process. Indicator or surrogate organisms were discussed, and it was concluded that there is presently no satisfactory indicator for the presence of parasitic organisms and that bacterial spores have potential for use as surrogates for treatment process effectiveness. Concerning the ability to assess risks, the workgroup indicated that there are a number of data gaps that need to be filled in. Analytical procedures for most of the parasitic organisms rely upon microscopic detection and identification which have inherent deficiencies in terms of time, expense, analyst experience, species identification and viability or infectivity determinations. There is very little reliable information on the removal and inactivation of parasitic organisms by animal waste treatment processes. Among the research needs the workgroup identified were development of better methods, data on pathogen release, transport and fate, and survival of parasites through treatment processes.

Participants in this workgroup were: Ronald Fayer, Leader; John Cicmanec, Facilitator; Susan Boutros; Norma Duran; Tim Evans; Walter Jakubowski; Joyce Perdek; Daniel Shelton; Gerald Stelma; Jeannette Thurston; and Scott Yates.

Parasitic Organisms

The discussion participants ranked the parasitic agents of concern in animal wastes in terms of their importance to public health. In arriving at their relative rankings by a consensus process, participants considered the following criteria: Potential risk to humans; Severity of disease; Availability of effective therapeutic treatment for infection; History of, and potential for causing, waterborne outbreaks; Occurrence in manure; and Survivability in the environment.

The ranking of the six parasitic agents deemed to be potentially significant to public health are shown in Table 1, column 2. *Cryptosporidium parvum*, and *Giardia lamblia* were assigned the highest priority (i.e., considered to be of greatest importance) because of their widespread occurrence in animal manures and the potentially severe illnesses they can cause. *Toxoplasma gondii,* microsporidia spp., and *Ascaris suum* (the only helminth considered to be of public health significance in the United States) were considered of intermediate importance to public health. *Toxoplasma gondii* only has a complete life cycle in cats. However, humans can become infected with the production of severe disease and two outbreaks of toxoplasmosis associated with water have been reported. Sources of microsporidia are unknown and infections occur primarily in immunocompromised populations. The significance of animal reservoirs for microsporidia with regard to human health is not known. *Ascaris suum* is highly prevalent in pigs and can cause human disease. *Balantidium coli* was assigned the lowest relative importance or significance to public health because, although it is highly prevalent in pigs, the incidence of infection in humans is low in the United States.

Indicator Organisms

Indicator organisms are used to determine the presence of microorganisms or to measure the effectiveness of a treatment. There are no known indicator organisms for detecting the presence of the organisms discussed in the previous section. There is currently no known relationship between the numbers of indicators and pathogens. *Bacillus subtilis* and *Clostridium perfringens* spores may be suitable indicators for treatment effectiveness. Spores of *Clostridium perfringens* and *Bacillus subtilis* have been used as surrogates of the

Table 1. Ability to Assess Risks from Parasites in Manure–Data Availability[a]

Organism (Known Animal Reservoirs)	Relative Importance to Human Health[b]	ID_{50} Data	Occurrence in Manure (Feces)	Transport	Survival	Detection & Methods
Cryptosporidium (cattle, pigs, sheep)	1	+++ (animal and human data)	+++	+	++	+
Giardia lamblia (cattle, pigs)	1	++ (animal and human data)	+++	+	+	+
Toxoplasma (cats)	2	+(animal data)	+++	+	+	Å
Microsporidia (cattle, dogs, cats)	2	0	0	+	0	+
Ascaris suum (pigs)	2	0	+	0	++	+
Balantidium (pigs)	3	0	+	0	0	0

[a]Relative amount of data available based on the experience and knowledge of the participants: 0 = little or no data available or of questionable quality; + = least amount of data available; ++ = intermediate amount of data available; +++ = greatest amount of data available

[b]Relative ranking of the organisms with regard to importance to human health based on a consensus of the workgroup (1 = highest; 2 = intermediate; 3 = lowest).

oocysts of *Cryptosporidium parvum* for purposes of environmental monitoring for the effectiveness of various wastewater and drinking water treatment processes (Rice et al., 1996). This application is based upon the relative size of the spores as well as their relative survival time in the environment which are roughly similar to *Cryptosporidium parvum* oocysts. However, there is insufficient information at this time to make a definitive statement. *Clostridium* may not be in high enough numbers to be a suitable indicator and it is not suitable for evaluating the composting treatment process. Survival and transport studies comparing bacterial spores and parasitic organisms are needed.

Ability To Assess Risks

Perhaps the greatest need for data as well as methodology development is in assessing the potential for risk of the various animal-borne parasitic diseases. For each parasite there can be more than one exposure pathway and there are many uncertainties for each step in these processes. A very challenging component of this process will be to develop a method or model that predicts how these organisms move through the environment, which includes runoff from pastures as well as movement through streams and rivers prior to potential human contact. Table 1 summarizes the consensus opinions of the workgroup participants regarding the data available for those factors that were considered important for conducting risk assessments.

In general, the group concluded that the greatest data deficiencies relate to transport mechanisms, infective dose, occurrence in animal manures, and survivability of the parasites under various conditions. Zoonotic transmission was also discussed. Animal-to-animal transmission of *Cryptosporidium, Giardia* and *A. suum* commonly occur and these routes are significant for dissemination of each of these organisms. The parasites multiply rapidly within the infected animal and this circumstance greatly increases the potential risk to public health. Within animal herds this can be controlled by frequent treatment with various chemotherapeutic agents. When these disease prevention measures are not practiced, infections with cryptosporidiosis and giardiasis can have serious economic impacts upon farmers.

The risk of transmission from vectors was also addressed. *Cryptosporidium* has been found on and in the digestive tracts of flies when housed with infected animals and the common house fly has been shown to

harbor *Giardia* and *Toxoplasmosis.* While Canada geese cannot actually be infected with *Cryptosporidium parvum,* they can play a role in passive transfer and serve as a vector. Mice and other rodents are possible vectors when they live in barns with infected animals. To date, 154 species of mammals have been reported to be infected with *Cryptosporidium* but it has not been established exactly how many various genotypes might be present and whether or not these organisms remain infectious for humans (Casemore et al. 1996).

Detection/Analytical Capabilities

Parasitic organisms have traditionally been detected and identified by microscopic examination of samples. While these procedures may be satisfactory for clinical specimens to establish a diagnosis, they have serious deficiencies for the examination of environmental samples. Concentration procedures, e.g., centrifugation or filtration, are generally necessary for environmental samples. Procedures such as flotation or immunomagnetic separation are used for separating the target organisms from debris in the sample. The concentrate is then examined by microscopy using stained or unstained preparations to enhance detection and identification of the organisms. Chemical, fluorochrome or fluorescent antibody stains may be used. The microscopic procedures have a number of disadvantages, e.g., they are time-consuming, experience intensive, insensitive and expensive. Positive identification to the species level is often not possible. In addition, they generally do not provide information on the viability or infectivity of the organisms detected.

Newer methods employing polymerase chain reaction (PCR) techniques have been developed for some of the protozoa. These have the capability for being very sensitive and identifying organisms not only to the species level but to genotype within the species. Methods indicating not only viability but infectivity of the parasites are needed in order to accurately determine the public health significance of their environmental occurrence.

Laboratory Procedures for the Detection of *Cryptosporidium* parvum

A number of direct microscopic techniques have been developed. The more widely used methods include Giemsa staining, Gram staining, Modified Kohn's staining, Methylene blue staining, Modified Ziehl-Neelson acid-fast staining as well as negative staining methods utilizing Carbol fuchsin and iodine. Fluorochrome stains that have been used include Acridine orange, Auramin O, and Auramine-carbol fuchsin. The more sophisticated immunofluorescence methods include both monoclonal and polyclonal antibody techniques and they have proven to be both very sensitive and very specific. None of these methods indicate the animal species of origin of the parasite or the infectivity. Also with the increased interest in cryptosporidiosis, flow cytometry methods and specific PCR genotyping procedures also have been developed. Tissue culture methods are now available for providing information on the infectivity of environmental isolates. The need does remain for standardization and validation for some of these methods.

Laboratory Procedures for the Detection of *Giardia lamblia*

Similar to *Cryptosporidium*, a wide variety of direct microscopic staining methods have also been developed for *Giardia*. To a limited extent, PCR techniques have been used for *Giardia* identification. Flow cytometry methods have also been used but with variable efficiency. To date the recovery percentages have been about 40-50% in a water matrix and around 1% from sludges. No suitable method for determining viability or infectivity is available.

Laboratory Procedures for the Detection of *Toxoplasma gondii*

Microscopic direct methods have been utilized for this protozoan parasite; mouse bioassay procedures also have been utilized. To date there have been no published reports for the detection of *Toxoplasma gondii* in environmental samples. Presently there are no molecular methods for the detection of *T. gondii* and all of the conventional methods still remain very time consuming, cumbersome, and expensive.

Laboratory Procedures for the Detection of Microsporidia

Conventional methods of microscopic examination for microsporidia can be used, however, special staining methods are needed, and because the spores are so small, a highly trained microscopist is needed. Immunofluorescence techniques are available but they are not as reliable as those available for other protozoan parasites. PCR methods are available for microsporidia, but so far they have been shown to have only limited application. Also, animal cell culture bioassays have been used but only for qualitative end points.

Laboratory Procedures for the Detection of *Ascaris suum*

The conventional flotation-separation procedures as well as direct fecal smears can be used for the diagnosis of *Ascaris suum* infections. These methods can be used quantitatively and they can be used to determine viability by observing embryonation within the ova, however, the methods are cumbersome and very time-consuming. The use of these methods does not differentiate *Ascaris suum* from *Ascaris lumbricoides.* Presently, molecular techniques such as reverse transcriptase-polymer chain reaction are not available for this parasite.

Laboratory Procedures for the Detection of *Balantidium coli*

Diagnosis for *B. coli* is performed using standard light microscopic techniques following separation using flotation procedures as well as directly from fecal smears. Using these basic procedures, semi-quantitative results can be obtained and to a trained microscopists eye, viability information is also possible. There are currently no known molecular or cultural tests available.

Processing/Control Technologies

Workgroup participants concluded that there is relatively little information available for this topic and that an extensive literature search should be conducted to determine the actual state of knowledge. Table 2 summarizes the participants' knowledge about data availability for the listed treatment processes. The group primarily drew information from the paper elsewhere in these proceedings by Fayer and Bowman. For *Ascaris* spp. mesophilic anaerobic digestion is considered to be the most effective method but it is heavily dependent on using higher temperatures, preferably higher than 40°C. Aerobic digestion can result in 100% inactivation within 4 days but again these processes are heavily dependent upon temperature. For *Ascaris* spp., alkaline stabilization can result in up to 100% inactivation but this process must occur above 40°C in order to be highly effective. Composting is adequate and lagoon storage has some effect.

For *Giardia* spp. that are present in an anaerobic sludge treatment process, there will be a 99.5% reduction in cyst numbers if the process is operated for greater than 10 days. The use of waste stabilization ponds will result in 99.9% removal of *Giardia* cysts and trickling filters have also been shown to result in a 98.3% reduction in concentration of cysts.

The only other specific body of data indicates that anaerobic digestion for 4 hours at 37°C will reduce the number of *Cryptosporidium parvum* oocysts by 90%.

Prioritized List of Research Needs

Participants developed a prioritized list of research needs by a consensus process. The development of improved methods for detecting and identifying these parasites was considered the most important need. Quantitative methods that are inexpensive, less experience intensive, rapid, validated and standardized would be desirable. The list of needs from highest to lowest priority is as follows:

- Develop improved quantitative detection and identification methods
- Determine the release, transport and fate of parasitic organisms in raw and treated animal manures

Table 2. Processing/Control Technologies–Data Availability[a]

Treatment	Cryptosporidium	Giardia lamblia	Ascaris suum
Anaerobic Digestion	++	+++	+++
Aerobic Digestion	Unknown	+++	+++
Composting	Unknown	Unknown	+
Lime Stabilization	Unknown	+++	++
Lagooning	Unknown	Unknown	+
Air Drying	Unknown	Unknown	Unknown

[a]+++ = most data available; ++ = some data available; + = little data available; Unknown = group unsure about how much was known about the technology for a particular organism

- Determine the survival, preferably with methods that indicate viability or infectivity, of parasites through animal manure treatment processes
- Develop improved management practices for animal manures
- Determine the prevalence of *Toxoplasma* oocysts and microsporidia spores in the environment
- Develop more information about the comparability of indicator/surrogate organisms for parasites in animal manures

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SECTION V

Microbial Risk Assessment

A Dynamic Model to Assess Microbial Health Risks Associated With Beneficial Uses of Biosolids

Pathogens in Biosolids - Microbiological Risk Assessment

Relevance of Microbial Risk Assessment for Foodborne Pathogens to Water Safety

A Dynamic Model to Assess Microbial Health Risks Associated with Beneficial Uses of Biosolids*

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Introduction

Biosolids are the finished solid products that are generated via the treatment of wastewater and subsequent treatment of sludge. Recently there has been increasing interest in the beneficial uses of biosolids, such as large-scale application to agricultural land as well as application at a smaller scale for use in home gardens. This rapid growth in the public use of biosolids has raised concerns about the abilities of the facilities that handle them to meet control and monitoring standards (Hay 1996, Logan 1995). Pollutants that may be present in biosolids are regulated by the U.S. EPA, under what is commonly known as the Part 503 rule. The sections of the rule that addressed chemicals were based on the results of risk assessments. The sections of the rule that addressed pathogens were not based on risk assessments "because methodologies had not been developed sufficiently to make such calculations"; rather they were based on performance or technology-based standards, management, and record keeping practices. (U.S.EPA 1995) The first step in addressing concerns related to microbial pathogens and biosolids is to provide a risk-based standard rather than a technology- or treatment-based standard.

In 1996, the U.S. EPA Office of Water contracted the International Life Sciences Institute to convene a panel of experts to develop a conceptual framework to assess the risks of human disease associated with exposure to waterborne pathogenic microorganisms. The panel acknowledged the need to account for unique aspects of infectious pathogens such as secondary transmission and immunity (ILSI 1996). Moreover, from a public health perspective, the probable number of people infected in an exposed population provides more insight and is more meaningful than the probability of individual infection. This public health perspective suggests the need for a population-based risk model.

One such risk model is based on disease transmission. In this document, consistent with the U.S. EPA microbial risk approach, the chemical risk model is extended to account for the unique properties of infectious disease processes using a disease transmission model. The principal approach taken in this research was to build upon a risk-based model that was previously developed for waterborne exposures (Eisenberg et al. 1996; Eisenberg et al. 1998; EOA 1999) and extend it to account for properties unique to biosolids and to exposure to pathogenic microorganisms from biosolids.

Model Development

The chemical risk paradigm (NRC 1983) has commonly been used as a generic framework for carrying out microbial risk assessments related to water- and food-borne pathogens (Crabtree et al. 1997, Farber et al. 1996, Sanaa et al. 2000, Voysey and Brown 2000). These assessments have typically focused on estimating the probability of infection or disease to an individual as a result of a single exposure event. The prob-

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*This updated paper is included in these proceedings with permission from Risk Analysis, a journal of the Society for Risk Analysis. An earlier version was presented by Eisenberg, et al. at the June 2001 Symposium.

ability that a susceptible individual becomes infected or diseased is a function of the dose of pathogens to which they are exposed; *i.e.*, when individuals in a susceptible state are exposed to pathogens from an environmental source, they move with a given probability to an infected or diseased state. This probability dose-response function is labeled P(d) in Figure 1. The dose is calculated by estimating two quantities, the concentration of pathogens at the exposure site and the rate at which susceptible individuals come into contact with the pathogen of interest. This dose quantity is then input into a dose-response function that estimates an individual risk value. The important health effects information required for the chemical risk model, therefore, is summarized in the function that represents this probability of infection, P(d), known as a pathogen-specific dose-response.

For both infectious and noninfectious disease processes, infectivity as a function of dose (estimated using a dose-response function) is an important factor to account for when estimating risk. There are, however, other factors that also potentially play an important role in infectious disease processes such as person-to-person transmission, immunity, asymptomatic infection, and incubation period. Each of these processes is discussed below.

To account for these additional factors when estimating risks associated with exposure to pathogenic microorganisms, a more sophisticated mathematical model than the one shown schematically in Figure 1 is required. The model shown in Figure 1 was extended to account for person-to-person transmission, immunity, incubation, and asymptomatic infection.

This extended model, shown in Figure 2, contains six disease states as compared to the two disease states presented in Figure 1. These six disease states are: 1) the susceptible state, S, defined as those who are susceptible to disease; 2) the exposed state, E, defined as those who have been exposed to pathogens but are asymptomatic and non-infectious; 3) the carrier state 1, C_1, defined as those who are infectious carriers but are asymptomatic; 4) the diseased state, D, defined as those who are symptomatic and infectious; 5) the carrier state 2, C_2, defined as those who were previously symptomatic but are now asymptomatic and infectious; and 6) the protected state, P, defined as those in a post-infectious state who are non-infectious and exhibit some level of immunity. Similar to Figure 1, the solid lines represent the movement of individuals from one disease state to another.

The rates of movement of individuals from one disease state to another are parameters represented in Figure 2 by Greek letters. For example five of these parameters represent processes that occur post-infection: 1) the rate of movement from the exposed state to an infectious state is represented by α where $1/\alpha$ corresponds to the latency period; 2) the rate of movement from the symptomatic infectious state to the asymptomatic infectious state is represented by δ where $1/\delta$ corresponds to the duration of symptoms during infection; 3) the rate of movement from the asymptomatic infectious state to the post-infection state is represented by both σ_1 and σ_2 where $1/\sigma_1$ and $1/\sigma_2$ correspond to the duration of infectiousness; and 4) the rate of movement from the post infection state to the susceptible state is represented by γ where,$1/\gamma$ corresponds to the duration of immunity. The reciprocal of these rates correspond to the duration in which an individual is in one of the following states: latently infected (E), asymptomatically infectious (C_1 and C_2), symptomatically infectious (D), or partially protected from further infection (P). These states can therefore be modeled mathematically using a set of delay equations (Eisenberg et al. In Press-b). An additional two parameters

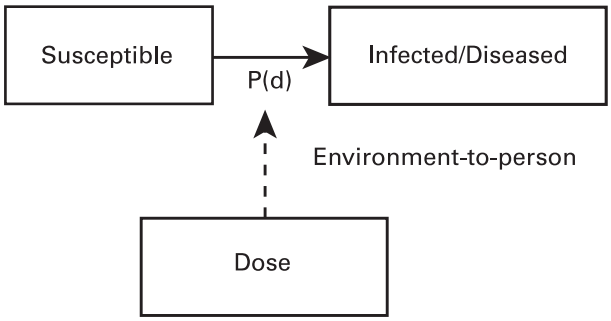

Figure 1. A schematic diagram of NRC chemical risk model applied to a microbial risk assessment, where d = dose and p(d) is the probability of infection.

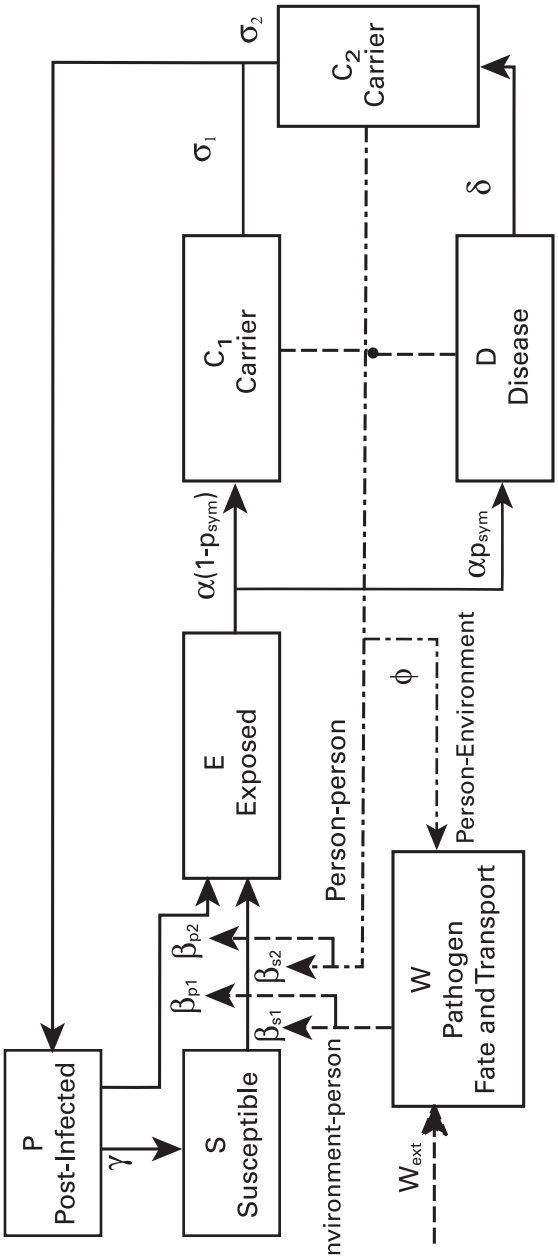

Figure 2. Disease transmission model (see text for parameter definitions).

complete the post-infection list of factors: p_{sym}, the proportion of symptomatic infections; and ϕ , the rate that infectious individuals shed pathogens into the environment.

In contrast, the transmission rate parameters, $\beta s1$, $\beta s2$, $\beta p1$, $\beta p2$ represent the infection event. The first subscript, *s* or *p*, denotes an infection event of a susceptible or partially protected individual, respectively. The second subscript, *1* or *2*, denotes whether the infection event occurs from environmental exposure or from person-to-person transmission, respectively. These transmission routes are represented by dotted lines in Figure 2. For instance, in one of the transmission routes, pathogens may move from infected individual to susceptible individual (labeled person-to-person transmission). This transmission route is described by $\beta s2$ and can be thought of as a product of two terms: a rate of contact between an infectious and susceptible individual; and a probability of infection given that a contact occurs. The parameter $\beta p2$ is analogous to $\beta s2$ except the transmission event occurs in an individual who is partially protected. Initially, individuals in state P are completely protected, i.e., $\beta p2$ is equal to zero times $\beta s2$. The protection decreases in time until the duration of protection has ended at which point $\beta p2 = \beta s2$ and individuals transition from the P (partially protected) state to the S (susceptible) state.

In the other transmission route, pathogens may move from an infectious individual to the environment and subsequently from the environment to a susceptible individual (labeled person-to-environment-to-person transmission). This environmental transmission route accounts for infected individuals shedding pathogens into the waste stream. The parameter ϕ characterizes the intensity of shedding from infectious individuals. These pathogens may eventually circulate back into the community through an environmental pathway (for example, through discharge of a wastewater treatment plant or via the generation and reuse of biosolids). This component accounts for the fate and transport of pathogens in the environment, as depicted in Figure 2, and as shown in more detail in Figure 3.

As shown in Figure 2, pathogens enter the environment either via infected individuals (persons from state C_1, C_2, or D) who shed ϕ pathogens per person per day, or via some external source, represented by W_{ext}. Pathogens remain in the system until they die off naturally, are eliminated by treatment or some other environmental process, or infect a susceptible/partially susceptible human host. The distribution of time that pathogens spend in the environment before being processed in a medium such as biosolids was modeled by a set of delay equations. This fate and transport process is characterized by two parameters, k_w,, the rate that pathogens move from contamination site to exposure site; and μ, the natural mortality rate during this process (Eisenberg et al. In Press-b). As shown in Figure 3, a fraction, *f*, of the pathogens are eliminated through some type of environmental processing. The parameter X_b represents this attenuation. As will be described in the next section, the case study presented in this manuscript adapted the model to the application of biosolids-amended soils. The attenuation, therefore, is the product of six biosolids process terms listed in Table 1. Those pathogens not in biosolids, the fraction 1-*f*, are processed through other means, such as drinking water treatment. The attenuation associated with these processes is represented by X_e. In our example, individuals are exposed to pathogens via biosolids by ingesting soil treated with biosolids, or via another non-biosolids environmental route. The parameters i_b and i_e are the biosolids and environmental ingestion rates respectively. This process results in a dose that an individual is potentially exposed and is estimated in this model as:

$$d = W[\, fX_b i_b + (1 - f)X_e i_e \,]$$

where W is the concentration of pathogens coming from the wastewater stream.

A Beta-Poisson dose-response function was used to determine the probability that an exposed person would become infected given a specific dose (Teunis et al. 1996):

$$P = 1 - (1 + \frac{d}{B})^{a}$$

where P represents the probability that an exposed individual will become infected, d represents dose of pathogen, and *a* and *B* are the parameters of the model that are identified when the function is fit to dose-response data. Once infected, individuals move from the susceptible state to the exposed state. The rate of this movement is defined as $\beta s1$ to distinguish the rate of environmental transmission from secondary transmission (person-to-person). The rate of movement of individuals from a partially protected state to the exposed state is similarly denoted by $\beta p1$ and is related to $\beta s1$ in the same manner as $\beta p2$ is to $\beta s2$.

The model structure presented in this manuscript is a general description of an infectious disease process, in that it accounts for a variety of properties, such as: 1) asymptomatic shedding of pathogens by those

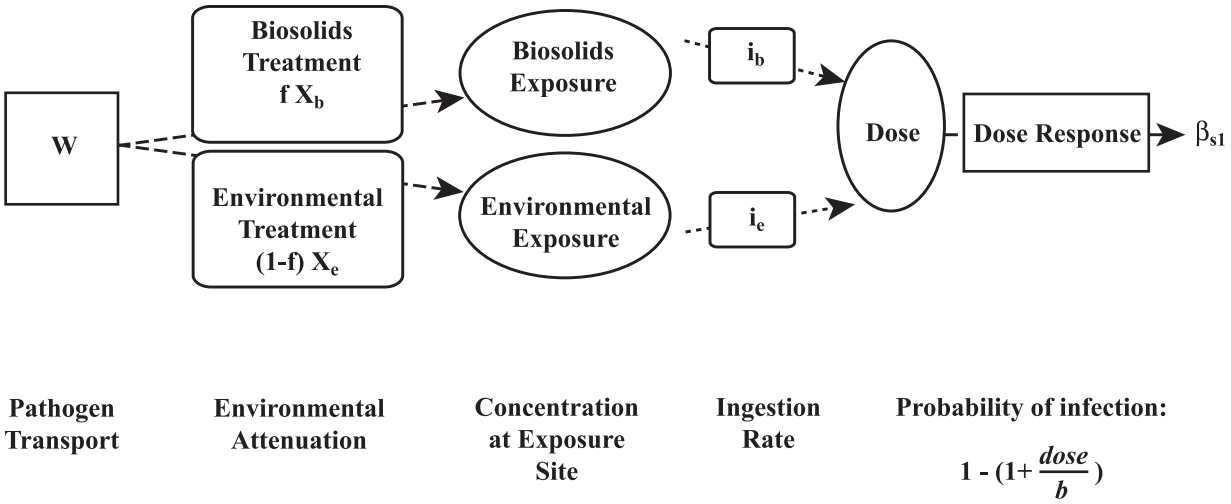

Figure 3. Pathogen fate and transport (environmental component of transmission model)

individuals that never present with symptoms; 2) asymptomatic shedding of pathogens by those individuals that present with symptoms but continue to shed pathogens for a period of time after symptoms cease; and 3) partial and temporary protection from reinfection. Not all infectious disease processes exhibit all of these properties and in general a transmission model structure can be simplified from the general structure presented here. We present a more general and complicated model structure in this manuscript to demonstrate the versatility of transmission models.

Methods

To illustrate the utility of this model, we chose a specific exposure pathway and pathogen. The exposure pathway chosen was direct ingestion of biosolids-amended soil. In general, the model described herein is applicable for all potential pathways of exposure to pathogens provided that the level of exposure can be characterized. We chose to focus on enterovirus due to its public health importance as well as the availability of data that were useful in identifying key model parameters. For this specific case study, we applied the disease transmission model to assess the risk in an endemic setting in which the pathogen is assumed to be already present in the human population. Alternatively, the model could be applied to assess the risk in an epidemic setting where the pathogen is newly introduced into a population (Eisenberg et al. 2002).

Scenario Definition

Risk characterizations are generally conducted for site-specific situations in which the environmental and behavioral conditions are well defined. To demonstrate this methodology, therefore, a scenario was developed that had a sufficient degree of realism to define these environmental and behavioral conditions.

Although the community characterized in this scenario was assumed to be closed to external sources of pathogens, *i.e.,* infectious community members generated pathogens in the environment, the model framework is flexible enough to relax this restriction. The community members were assumed to be homogeneous with respect to exposure and susceptibility to disease (again, extensions of the model could permit estimation after relaxing these assumptions). With respect to the pathogen, enteroviruses were assumed to be endemic in the community and the only source of enterovirus pathogens in the environment was assumed to come from infectious individuals. In addition, the virus population was assumed to be homogeneous, *i.e.,* distinct characteristics of the different enterovirus strains were not modeled.

Pathogens were assumed to be transported to the biosolids exposure site via either the wastewater stream from within the community, or from an external source from outside of the community. Through this transport the pathogens were attenuated by a wastewater treatment facility, natural die-off, and dilution. Upon reaching the biosolids processing stage it was assumed that Class B biosolids were generated; *i.e.,* wastewater sludge underwent mesophilic anaerobic digestion for 20 days at 35°C and was then dewatered to 20% solids and air-dried for 3 months.

With regard to application, it was assumed that biosolids were land-applied one time, and that no other sources of biosolids exposure existed; *i.e.* community members could only be exposed to biosolids by ingesting treated soil. The units of the state variables were in fractions; i.e., S = 0.4 reflects that 40% of the population is in the susceptible state. An estimate of the population size is therefore not required.

Biology and Epidemiology of Enteroviruses

This section provides a rationale for the specific choices of parameter values presented in Tables 1 and 2 and discussed in the following section.

Epidemiology

The enterovirus genus comprises 64 serotypes including poliovirus, coxsackie A and B viruses, echoviruses, and enteroviruses. Infection can be spread by direct person-to-person transmission (Melnick 1996), usually through ingestion (Feachem et al. 1981), however, exposure through inhalation of aerosols also occurs (Morens and Pallansch 1995). Infection can also be spread by indirect transmission (person-to-environment-to-person) mediated through food, water, or other environmental sources. Enteroviruses have been found in surface waters, ground waters and sewage (Albert and Schwartzbrod 1991) as well as in sludge (Sorber and Moore 1986). Numerous outbreaks have be associated with both direct and indirect transmission (Hawley et al. 1973, Kogon et al. 1969, Pichichero et al. 1998, Reintjes et al. 1999, Torphy et al. 1970). In tropical climates, the circulation of enteroviruses is year round whereas in temperate climates, infections typically occur in the summer and fall (Moore 1982). Enteroviruses cause a wide range of diseases from mild rashes and cold-like symptoms to more serious meningitis and encephalitis (Moore 1982). A high percentage of infections, however, are subclinical (Kogon et al. 1969). Most data suggest that over 50% of infections are asymptomatic (Couch et al. 1969, Hall et al. 1970, Kogon et al. 1969). The incubation period, time from infection to the presentation of symptoms, is generally 2 to 10 days (McAllister 1960) and shedding times can range from a day to several weeks but are generally less than 5 days (Couch et al. 1969, Kogon et al. 1969, Melnick 1996). Shedding has been observed to continue even after symptoms cease (Kogon et al. 1969). The literature generally supports the fact that enteroviral antibodies are an indicator of partial and temporary protection to either infection or disease. Protection due to prior exposure has been

Table 1. Summary of parameters used to estimate the environmental transmission parameter, βs1

Parameter Description		Low estimate	Middle estimate	High estimate
W_{extb}	Concentration of virus in sludge, from external source (PFU/g)	0	0	0
W_{exte}	External virus from environment (PFU/g)	0	0	0
μ	Pathogen die-off (1/day)	0.033	0.066	0.166
k_w	Rate of movement of organisms from contamination site to exposure site (1/day)	0.133	0.266	0.666
f	% pathogens from W present in biosolids	0.1	0.25	0.5
i_b	Ingestion rate of treated soil (g/day)	0.001	0.01	0.2
i e	Ingestion rate through environmental routes (1/day)	2	2	2
a	Dose-response parameter	0.126	0.26	0.5
B	Dose-response parameter	.21	.42	.84
X_e	Environmental attenuation factor	0.0001	0.01	0.1
X_b	Biosolids attenuation factor (computed as the product of the 6 parameters listed below)	0.0001	0.01	0.1
r	Treatment reduction. Log removal due to treatment.	0.01	0.05	0.1
s	Storage reduction rate. Reduction rate due to storage of biosolids prior to application (1/day)	0.9985	0.9988	0.9992
t_s	Time of storage prior to land application (days)	90	90	90
a	Application reduction. Reduction factor to account for application methods	1	1	1
1	Die off rate (1/day)	0.1	0.237	0..562
t_e	Time to exposure (days)	365	7	1

Table 2. Summary of parameters associated with the disease process for enterovirus

Parameter Description		Low estimate	Middle estimate	High estimate
α	Transition rate out of exposed state. Inverse of incubation period. (1/day)	0.2	0.4	2
δ	Transition rate out of diseased state. Inverse of disease duration. (1/day)	0.2	0.8	4
σ_1	Transition rate out of carrier state. Inverse of duration of shedding. (1/day)	0.066	0.4	2
σ_2	Transition rate out of carrier state. Inverse of duration of shedding. (1/day)	0.066	0.4	2
γ	Transition rate out of post infection state. Inverse of duration of immunity (1/day)	0.008	0.04	0.4
P_{sym}	Probability of symptomatic response.	0.25	0.5	0.75
ϕ	Intensity of shedding (pathogens/day)	10^4	10^6	10^8
β_{s2}	Transmission rate (susceptible to exposed from person-to-person contact) ([infected persons contacted]/day)	0.01	0.05	0.1
β_{p2}	Transmission rate (partially protected to exposed from person-to-person contact) ([infected persons contacted]/day). Computed as a fraction of β_{s2}			

observed both experimentally (Schiff et al. 1984) and in observational studies (Hall et al. 1970; Kogon et al. 1969). Data suggests that the level of antibodies tends to decrease over a period of time that ranges from less than a year to several years (Aoki and Sawada 1992).

Survival

Enteroviruses can readily survive in the environment. The half life in soil can range from 5 or 6 days to 30 days depending on various environmental factors such as temperature (Sorber and Moore 1986). The reduction rate in biosolids has been observed from 0.08 to 0.02 $\log_{10}$s reduction per day, again depending on a variety of environmental factors (Ahmed and Sorensen 1995, Ahmed and Sorensen 1997). Reduction due to different treatment processes, such as mesophilic anaerobic digestion can range from 1 to 4 $\log_{10}$s (Ahmed and Sorensen 1997, Feachem 1983, Sorber and Moore 1986, Tata et al. 2000).

Dose

The dose to which an individual is exposed depends not only on the concentration of pathogens at the exposure site, but also on the ingestion rate. The U.S. EPA has assumed that 95% of children ingest 200 mg soil/day or less for exposure assessment purposes (U.S.EPA 1989). Data from dosing trials (Schiff et al. 1984) and subsequent analysis of these data is used in our model to describe the dose response relationship (Teunis et al. 1996).

Parameterization

The parameter values used in the simulation study are shown in Tables 1 and 2. A low, medium, and high value was assigned for each parameter based on information presented in the literature. Additional details on how these values were determined are provided elsewhere (Eisenberg et al. 2003-b).

The eight parameters in Table 2 are related to the disease process of the pathogen within the human host. The first five parameters represent the post-infection processes described in the previous section. Three parameters related to the infection processes: the proportion of infections that are symptomatic, p_{sym}, and shedding rate of infectious individuals, ϕ, as well as the person-to-person transmission rate parameters, $\beta s2$ and $\beta p2$ are also shown in Table 2. Whereas most of the parameters were based on information from the literature, the values for ϕ, $\beta s2$, and $\beta p2$ were chosen to explore the behaviors of the model. In this manner the simulation analysis was designed to identify the conditions for which the different transmission processes were important in determining risk.

The remaining parameters are environmental factors that are all summarized into a dose value that is in turn used to identify the environmental transmission rate, $\beta s1$. The first two parameters shown in Table 1 represent external sources of pathogens. These parameters are set to zero for this case study, since the scenario chosen was a closed community in which all pathogens in the environment come from the community. The next two parameters pertain to the fate and transport of the pathogen from contamination site to exposure type. The values chosen were set to explore a range of possible transport times, from 6 to 30 days, as well as a range of mortality rates that correspond to average life spans from 6 to 30 days.

The next seven parameters are required to estimate the probability of infection from the environment, where the dose that an individual is exposed to is estimated to be

$$d = W[\, fX_b i_b + (1 - f)X_e i_e \,],$$

where W is the concentration of pathogens coming from the wastewater stream, f is the proportion of pathogens that enter the biosolids stream, X_b is the attenuation of pathogen concentration caused by the biosolids process, X_e is the attenuation of pathogen concentration caused by all the other environmental pathways besides biosolids, and i_b and i_e are the biosolids and environmental ingestion rates respectively. The biosolids attenuation parameter, X_b, was modeled in more detail as the product of the last six parameters shown in Table 1. These values were chosen using information from the literature. Likewise, data from the literature was used to identify the two ingestion rate parameters. Little is known, however, about the remaining two parameters, f and X_e. For this reason, parameter values were chosen that would allow for the exploration of their role in affecting risk estimates. The dose is then used as an input variable of a dose-response function, which in turn is used to estimate $\beta s1$.

Measure of Risk

Cumulative incidence (CI) was used to measure disease occurrence or morbidity. Cumulative incidence was defined as the number of new cases of disease during a one-year period divided by the total population at risk. For example, if a population of 1,000 people is at risk of developing disease and 15 people actually develop it over the time span of one year, the resulting CI would be 15/1000. This is analogous to the risk number used in more traditional risk assessments and can be interpreted as an annual per person risk of 0.015. The CI measure can therefore be interpreted as a risk estimate. In the model, the cumulative incidence was calculated by summing (or integrating) the number of new cases entering into state D at every time step.

For each parameter combination, two simulations were run. The first was to simulate the condition in which biosolids exposure was present (CI_b). The second was to simulate the condition in which biosolids exposure was absent (CI_o). This second condition was accomplished by setting the parameter X_b equal to zero. To calculate the number of cases that were due to biosolids, known as the attributable risk, CI_0 was subtracted from CI_b.

To illustrate this risk measure, two examples are presented, one illustrating a condition in which AR_b is low and one illustrating a condition in which AR_b is high. In the first example, parameter values were chosen to simulate a condition in which biosolids exposure was relatively low and secondary transmission was high. When simulated with biosolids present, the model produced a one-year cumulative incidence of 2.61 cases per person per year; i.e., on average, each individual is symptomatic 2.61 times per year. When simulated with biosolids absent, the model produced a one-year cumulative incidence of 2.58 per person per year. Therefore the attributable risk associated with biosolids in this hypothetical example was AR_b = 0.03 (i.e. 2.61 – 2.58) cases per person per year. The fact that the baseline incidence of the pathogen was high suggests that in this hypothetical example the pathogen was highly infectious and is able to move through the population effectively through person-to-person transmission. The fact that the attributable risk was low suggests that only a small fraction of those infections is due to actual biosolids exposure.

In an alternative example, parameter values were chosen to simulate a condition in which the exposure to biosolids was high and secondary transmission was low. The results of the model simulation produced a one-year cumulative incidence when biosolids were present equal to 0.83 cases per year per person and a cumulative incidence of 0.33 per person per year when biosolids were absent. Therefore the attributable risk associated with biosolids in this hypothetical example was AR_b = 0.5 cases per person per year.

This attributable risk measure is equivalent to that used in a more traditional analysis (one that ignores person-to-person transmission). Using the chemical risk paradigm, concentrations of the pathogen at the

exposure site are measured and then used to estimate a dose. A dose-response function is used to estimate the risk associated with exposure to biosolids. This risk estimate accounts for the direct risks due to ingestion of a given dose of pathogens. Using a disease transmission model the risk associated with biosolids exposure is also estimated. The difference is that this risk is based on not only the direct risk associated with an individual ingesting pathogens from the environment, but also on the indirect risks that are due to the propagation of the pathogen through person-to-person transmission.

Simulation Approach

At the beginning of each simulation, 95 percent of the population was placed in the susceptible state and the remaining 5 percent were placed in the exposed state. Each simulation was run for 1,500 days to ensure that steady state was reached; i.e. the number of people in each state reached equilibrium. After 1,500 days had elapsed, the number of disease onsets for a span of 1 year was counted. The risk estimate, therefore, corresponded to a 1-year incidence associated with enterovirus disease in an endemic setting.

A sensitivity analysis was conducted to determine which parameters in the model impacted AR_b the most; i.e., to determine the parameters to which the output was most sensitive. The following ten parameters were included in the sensitivity analysis: X_b Attenuation of pathogens in biosolids; X_e Attenuation of pathogens in the environment (other than biosolids); f Fraction of environmental pathogens that are in biosolids; σ Rate of loss of infectiousness; α Rate of moving from infected to infectious; $\beta s1$ Secondary transmission rate; B Dose response parameter; a Dose response parameter; γ Rate of decline in immunity; ϕ Shedding rate.

Each parameter was assigned a high, medium, or low value from the available literature (see Tables 1 and 2). Parameters not involved in the analysis were assigned a medium value. Simulations were conducted for each possible combination of parameters. This resulted in a total of 3^{10} (59,049) simulation runs. For each simulation, AR_b was calculated. Thus, each unique set of parameters had a corresponding AR_b. A Classification and Regression Tree analysis (CART) was used to classify the outputs into low and high AR_b (Breiman et al. 1984, Eisenberg et al. 1996, Eisenberg et al. 2003-b). The results of the CART analysis were summarized in a tree diagram that was used to prioritize the importance of the model parameters in determining the conditions under which AR_b was high or low.

Results Of Case Study

Sensitivity analysis of our simulations identified four factors as being the most important in determining the level of risk attributable to biosolids (AR_b). The first factor of importance was the relative contribution of biosolids towards exposure, compared to all other environmental pathways. In our model, this is represented as the ratio of X_b to X_e. The parameter X_b represents the percent reduction in pathogens, due to a variety of biosolids related processes and X_e represents this reduction for all other environmental sources other than from biosolids; e.g. an X_b value of 0.01 indicates a 2 log reduction of pathogens during biosolids processing whereas an X_b value of 1 indicates no reduction at all. The parameter f represents the fraction of all pathogens in the environment that enter into the biosolids stream (Figure 3). If, for a given value of f, X_b is small (where smaller values correspond to greater attenuation) compared to X_e then a greater proportion of pathogens comes from environmental pathways other than biosolids. Under these conditions, the biosolids pathway has a minimal contribution to the disease incidence and therefore the attributable risk associated with biosolids (AR_b) is small. The relative contribution of biosolids also depends on f. This relationship comes from comparing the contribution of biosolids to the total dose of pathogens that an individual is exposed to, WfX_b, with the total dose of pathogens associated with other environmental pathways, $W(1-f)X_e$. The relative contribution of biosolids can be then summarized by

$$\frac{fX_b}{(1-f)X_e}.$$

Second in importance was the rate of shedding from infectious individuals. Rate of shedding, ϕ, was defined as the number of pathogens shed per person per day. Only moderate values of ϕ resulted in an increased AR_b. When shedding was very high or very low, biosolids were not a factor in disease incidence.

The rate of person-to-person (secondary) transmission was a third factor impacting AR_b. This was represented by $\beta s2$ in the model. When $\beta s2$ was high, person-to-person transmission accounted for a greater proportion of infections. Under this condition, competing transmission pathways, such as those associated with biosolids and environmental exposures, account for fewer infections. As a result, AR_b was low.

The fourth factor found to affect AR_b was immunity. Movement from the protected state to the susceptible state was governed by γ. Duration of immunity was inversely proportional to γ; i.e. for small values of γ, duration of immunity is high. Shorter periods of immunity led to higher values for AR_b while longer periods of immunity led to smaller AR_b values.

The hierarchy of importance exhibited by these factors is important. As can be seen in Figure 4, the impact of each successive factor is conditional on the existence of the preceding factor; i.e. secondary transmission influences AR_b but only if shedding is moderate and the relative contribution of biosolids towards exposure is high. In fact, if any of the following conditions were true — the contribution of biosolids towards exposure was small compared to the contribution from other sources; the rate of pathogen shedding was relatively high or low (not moderate); person-to-person transmission was relatively high — then AR_b was low regardless of other parameter values.

Relative Contribution of Biosolids Towards Exposure

The most important parameters affecting the attributable risk associated with biosolids, AR_b, were X_b, the attenuation of pathogens in the biosolids, and X_e, the attenuation of pathogens in the environment other than biosolids. We found that if X_e was large compared to X_b then AR_b was low. In this case, biosolids treatment was more efficient at eliminating pathogens than other treatment processes such as those associated with water or food. As a result, most pathogens at exposure sites came from sources other than biosolids. Conversely, if X_b was either comparable or large compared to X_e, i.e. biosolids treatment was less efficient, then AR_b had the potential to be high depending on other parameter values. Based on our simulations, the average AR_b for all simulations where X_e was set to a medium or high value was only 0.001 cases/person/year. When X_e was set to a low value and X_b was set to a medium or low value, the average AR_b for all simulations increased about ten-fold to 0.013 cases/person/year. When X_e was low and X_b was high, the average AR_b increased ten-fold yet again to 0.134 cases/person/year. Clearly, more work is needed to provide rigorous estimates of these parameter values; however, these simulations illustrate the basic property that both the relative amount of pathogens diverted to biosolids vs. other environmental exposure sites, and the relative attenuation of the different environmental processes compared with biosolids can be important determinants of risk.

To provide a better understanding of these results, the process of obtaining these risk estimates can be compared to a more traditional approach to estimating risk. In a more conventional microbial risk assessment, based on the chemical risk paradigm, AR_b would be estimated by collecting data at the exposure site to estimate dose, and in turn using this dose estimate as input to a dose-response function to estimate risk. This approach provides an estimate of the attributable risk associated with biosolids exposure. The basic assumption is that there is no interaction between biosolids exposure, human shedding of pathogens, person-to-person transmission, and environmental (other than biosolids) exposure. Given no interaction, this risk estimate is simply the first term of the attributable risk difference used in this approach. Biosolids attributable risk is the difference in risk when all exposure pathways are present minus the risk when the

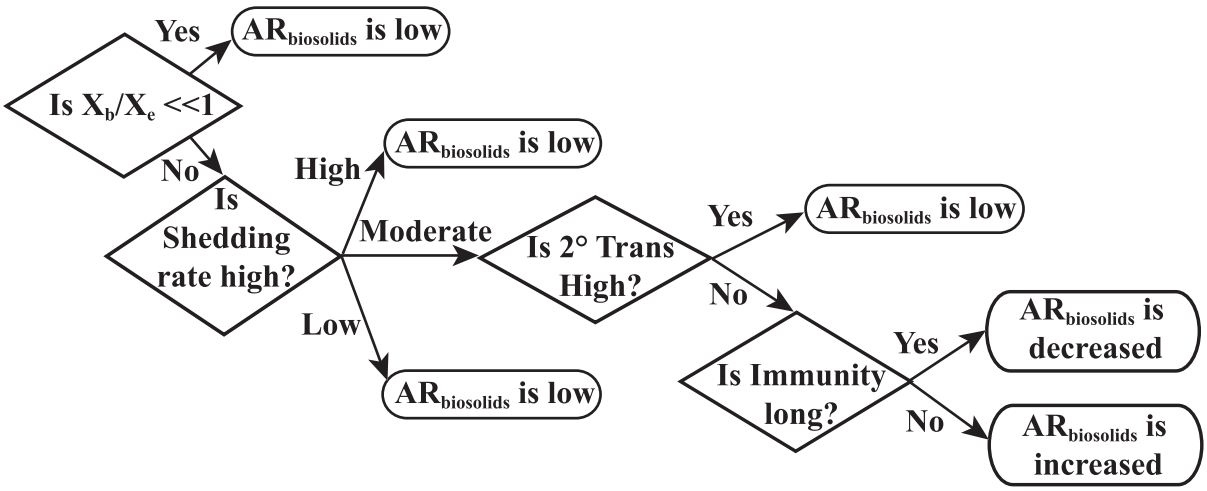

Figure 4. Decision tree for classifying the attributable risk associated with biosolids (AR_b) as high or low

biosolids pathway is eliminated. The traditional approach assumes that the second term (the risks when the biosolids pathway is eliminated) is zero. That is, the risk attributable to biosolids is simply the direct risks coming from exposure to biosolids. By incorporating these interactions, as demonstrated in the transmission model presented for this study, the risk estimates associated with biosolids exposure are achieved by looking at the difference between the risk when biosolids are present and when biosolids are absent. In this way risk becomes a function of an additional set of factors not accounted for in the chemical risk paradigm.

In our scenario, low, medium, and high values of X_b were set to 0.0001, 0.01, and 0.1 respectively. It was found that anytime X_b was set to 0.0001, AR_b was approximately zero regardless of other parameters, however when X_b was 0.01, conditions existed in which AR_b could become significant (e.g. when X_e was low). Thus, in our scenario reducing X_b to 0.0001 is desirable because it virtually eliminates disease attributable to biosolids. In this model it was assumed that the attenuation of pathogens within biosolids is estimated by the product of each biosolids process being modeled. The log transformation of the parameter X_b provides an *estimate of treatment efficiency* in units of log removal. The log removal, therefore, is computed by the following equation:

$$\mathbf{1n}(X_b) = \mathbf{1n}(r \cdot s \cdot t_s \cdot a \cdot l \cdot t_l)$$

where r = treatment reduction, s = storage reduction rate, t_s = storage time, a = application reduction, l = die-off rate, and t_e = time to exposure; a practical way to reduce X_b would be to increase storage time.

One caveat to examining the ratio of X_b and X_e is that the results presented here are based on the assumption that f, the fraction of environmental pathogens associated with biosolids, is known. In general, the expression for the relative contribution of biosolids to environmental exposure to pathogens should include the ratio f / (1-f), as shown above. Alternatively, the interpretation of X_b and X_e could include the factor f.

Shedding

The only known source of enterovirus pathogens is from human hosts. The mechanism from which pathogens are present in the environment, therefore, is the shedding of the virus by infectious individuals. One potential pathway consists of pathogens entering the wastewater stream and being transported into biosolids. In our model, the shedding process is represented by the parameter ϕ, the shedding rate of pathogens from infectious individuals into the environment. The parameter, ϕ, is defined as pathogens shed per infectious individual per day. Analysis of our simulations suggest that the rate in which pathogens are shed becomes important if X_b is not << X_e. That is, shedding plays a role in biosolids associated risk only if pathogens are present in biosolids in sufficient quantity relative to other environmental sources. Under this condition, therefore, shedding may play a role in the level of risk associated with biosolids exposure.

Specifically, our analysis suggested that the attributable risk due to biosolids exposure, AR_b, may be large if the shedding rate is a moderate value (in our model a 'moderate value' was somewhere between 10^5 and 10^7 pathogens/person/day). To understand this relationship better we can examine the effects of shedding on both the incidence of disease and the risk attributable to exposure to biosolids. In this case study, all infections ultimately came from other infectious individuals; i.e., pathogens did not come from outside of the community. Based on this assumption, low rates of shedding resulted in an estimate for incidence close to or at zero (in our model a 'low value' was below 10^5 pathogens/person/day). That is, there were not enough pathogens shed into the environment to sustain transmission via environmental exposure. Another way to view this result is to observe that the pathogens released into the environment are attenuated through dilution, natural die-off, or treatment. Only a fraction, therefore, makes it to an exposure site in which an individual may ingest a pathogen and become infected. If the number of pathogens released by infectious individuals during its infectious period results, on average, in less than one case of infection through environmental exposure, then the disease will not persist through this environmental loop alone. For example, in our simulation, if 100 infectious individuals shed pathogens into the environment at a rate of 10^4 pathogens/person/day, then these pathogens will potentially result in further infection. If, however, all the pathogens shed into the environment by these 100 infectious individuals during their duration of infectiousness produce less than 100 infections then the pathogen cannot persist through this environmental pathway. Given this zero estimate for incidence, the attributable risk was also zero.

When the shedding rate was set to a high value, the simulation results suggested that the environmental exposure was also high, resulting in a large incidence estimate. The incidence estimate of 3.5 cases per

person per year (Figure 5a) may be unrealistically high, suggesting that either the shedding rate estimate was too high or attenuation estimates were too low. For the purposes of this investigation, however, our concern was to demonstrate the potential relationships between model parameters and risk and not produce a precise estimate of risk. This high rate of shedding resulted in a high rate of exposure from all environmental exposures. Any additional contribution from biosolids was insignificant. Therefore, AR_b was close to zero for high values of ϕ (Figure 5b).

Figure 5a shows disease incidence when biosolids were present and when biosolids were absent. As just observed, the difference between these two plots (AR_b) was negligible at high values and low values of ϕ. However, at moderate values of ϕ there was a noticeable difference. For example, when $\phi = 10^4$ the number of cases per person per year was the same (zero) regardless of biosolids presence. Similarly, when $\phi = 10^8$, the number of cases per person per year was approximately 3.5 with or without biosolids. However, for $\phi = 10^6$, the number of cases per person per year was 2.3 when biosolids were present compared to 2.0 when biosolids were absent. The difference between the two scenarios is plotted in Figure 5b. The difference reached a maximum at approximately 10^5 and decreased for higher values of ϕ. Our analysis revealed that, given X_b is not << X_e, the average AR for all scenarios where ϕ was set to a medium value was 0.33 cases/person/year. When ϕ was set to a high or a low value, AR_b was only 0.036.

The suggestion that shedding rates may affect risk estimates of exposure to biosolids emphasizes the fact that enteroviruses present in biosolids originate from human sources. These pathogens are transported through the environment from contamination site to exposures site. Many potential control points exist along this environmental pathway that may help to attenuate the concentration of pathogens and therefore decrease the risks associated with exposures to biosolids. The introduction of shedding into the risk assessment highlights how the different components of an infectious disease process interrelate in estimating risk.

Secondary Transmission

Secondary transmission was defined as the transmission of a pathogen from an infectious person to a susceptible person. This may occur within communal settings such as a household or day care center, in which the transmission event occurs through an intermediary fomite such as a sink or toilet. Figure 6a suggests that as secondary transmission increases the incidence of disease within the community increases. Therefore, secondary transmission can serve to amplify an index case that is obtained from an environmental source, both those associated with biosolids and those associated with other sources. The two curves shown in Figure 6a were compared to assess the importance of secondary transmission to risks associated with biosolids. The difference between these two curves, shown in Figure 6b, provides this estimate of the attributable risk associated with biosolids exposure for different levels of secondary transmission. Again,

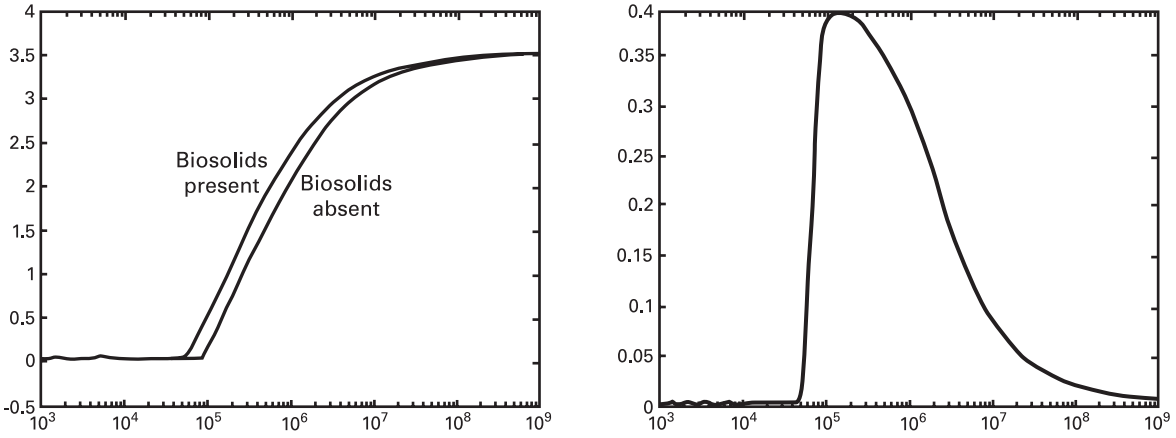

Figure 5a and 5b. a) Disease incidence as a function of ϕ, shedding rate, when biosolids were present and when biosolids were absent; b) Attributable risk (excess risk) associated with biosolids exposure (the difference in the two incidence curves shown in a). Plots are based on the condition that the ratio, X_b / X_e is not << 1. All other parameters, besides ϕ, were set to medium values.

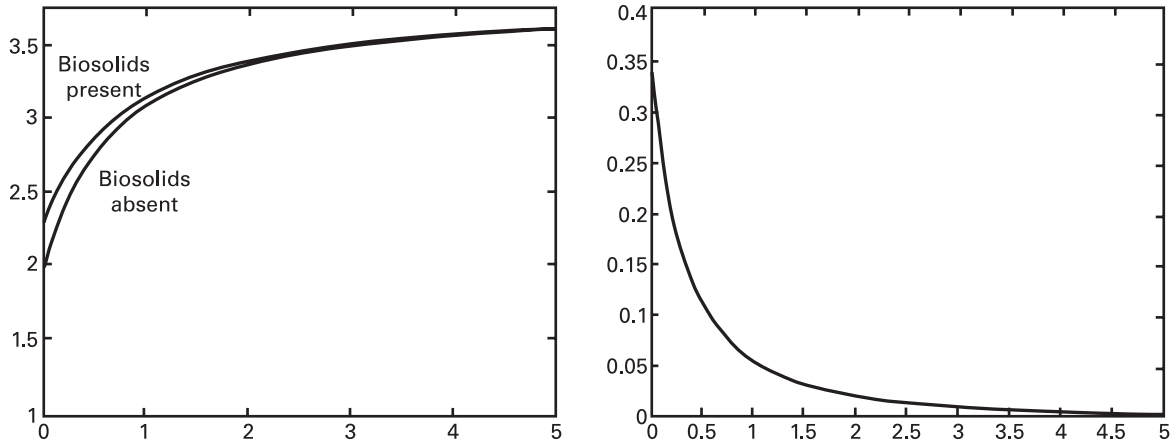

Figure 6a and 6b. a) Disease incidence as a function of β_{s2}, the rate of secondary transmission, when biosolids were present and when biosolids were absent; b) Attributable risk (excess risk) associated with biosolids exposure (the difference in the two incidence curves shown in a). Plots are based on the condition that the ratio, X_b / X_e is not << 1 and the shedding rate is "moderate" (see text for details). All other parameters, besides the transmission rate β_{s2}, are set to medium values.

estimating the biosolids associated risk in the chemical risk paradigm is accomplished by simply measuring the concentration of biosolids at the exposure site. This assumes that other transmission pathways, such as secondary transmission, are zero. Once we account for these other transmission pathways, the appropriate risk estimate becomes the difference in incidence between the condition in which there is no biosolids exposure and the condition in which there is biosolids exposure.

As summarized in Figure 4, secondary transmission was not an important determinant of biosolids associated risk unless the relative contribution of biosolids vs. other environmental sources was comparable or high (X_b was not << X_e), and the shedding rate, ϕ was "moderate" (between 10^5 and 10^7 pathogens/person/day). Under these conditions, increased person-to-person transmission serves to dilute the impact of other competing transmission pathways and drives the value of AR_b down; i.e. when person-to-person transmission is high, the likelihood of developing disease from an environmental pathway is small. Figure 6b shows the difference between these two scenarios (AR_b). The difference is largest when secondary transmission is zero. At this point, all disease is attributable to environmental exposure sources. As $\beta s2$ increases the two plots converge and the difference approaches zero. Note that when $\beta s2$ is large ($\beta s2 > 4$), AR_b is small (~ 0), however the incidence of disease in the community is large (~ 3.5).

The finding that secondary transmission serves to decrease the attributable risk associated with biosolids is dependent on the fact that we are examining a single community endemic condition in which biosolids are one of many environmental sources of a given pathogen. There may be other conditions, such as the case in which biosolids serves as a potential risk to a point source outbreak of a unique viral strain that has no other environmental sources. Under these conditions, secondary transmission may serve to amplify not only the overall incidence of disease within the community, but also the risk associated with biosolids exposure.

Immunity

After an individual recovers from an infectious period, our model allows for that individual to be partially and temporarily protected from further infection. The average time of protection is measured by the inverse of the parameter γ ($1/\gamma$). During this period, $1/\gamma$, the level of protection, is assumed to linearly decrease from full protection to no protection. This conferred immunity serves to limit the number of individuals at risk at any given time. The smaller the duration of protection, the greater the number of infections possible in any given year. This is illustrated in Figure 7a where the cases per person per year decrease with increasing duration of protection. Conversely, as the duration of protection increases, a larger proportion of the population is in the protected state and a lower proportion is completely susceptible. Under these conditions, the overall incidence in the community is low. If the overall risk of disease for a particular pathogen is low, then the attributable risk associated with biosolids will also be low. Long durations of immunity (small γ values), therefore, lowered the attributable risk associated with biosolids, AR_b. As can be seen in Figure 7b, the difference in disease incidence when biosolids were present vs. when

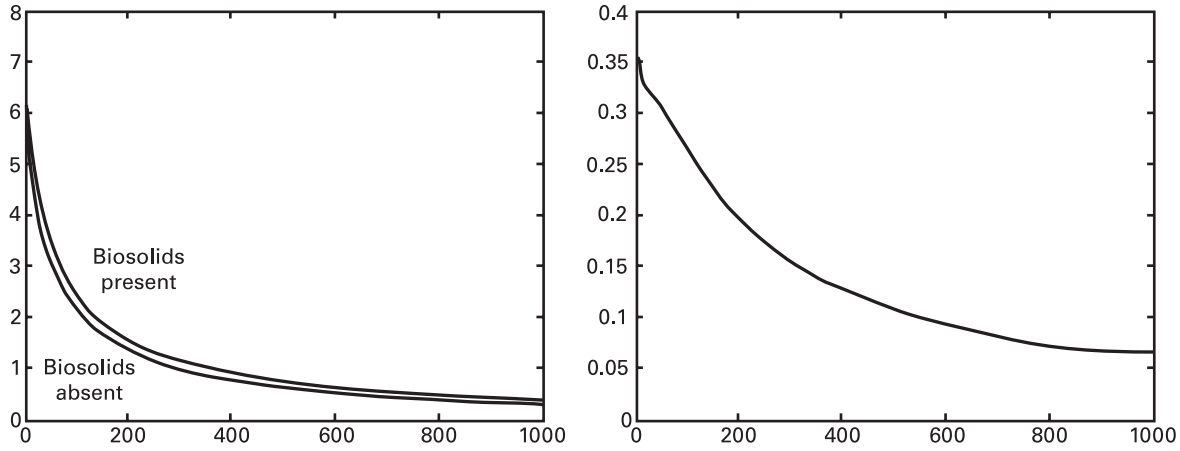

Figure 7a and 7b. a) Disease incidence as a function of 1/γ, the duration of immunity, when biosolids were present and when biosolids were absent; b) Attributable risk (excess risk) associated with biosolids exposure (the difference in the two incidence curves shown in a). Plots are based on the condition that the ratio, X_b / X_e is not << 1, the shedding rate is "moderate" (see text for details), secondary transmission is negligible. All other parameters are set to medium values.

biosolids were absent is greatest for short durations of immunity. This difference decreases as duration of immunity increases.

The model structure used in this study assumed that enteroviruses were a homogeneous group of viruses in which exposure to one strain confers protection from infection of any other strain. Although some degree of cross immunity between strains is plausible, it is likely that there is not complete cross immunity across all strains. Further work is needed to examine the effects of different levels of cross immunity.

A Decision Tree for Estimating Risks

Figure 4 provides a decision tree for determining the level of risk attributable to biosolids exposure. The first decision node shown in the figure addresses the environmental exposure component of the risk model. It is at this level that risk managers can examine the effects of treatment process options to ascertain the costs of ensuring a low attributable risk. Different processing options, therefore, can be evaluated to determine whether or not a given change in the process will have an effect on the first node decision. The decision at this node is whether or not the contribution of biosolids exposure is significant compared with the contribution of other sources. The actual determination of when, for example, $X_b / X_e << 1$ is a risk management decision that is dependent on the level of tolerable risk. An example examining the increase of storage time was discussed earlier.

If the decision at the first node is no, our simulations suggest that the shedding rate of the pathogen into the environment, the secondary transmission rates, and the duration of protection after being exposed and infected, are important factors that may result in low attributable risk.

Decision trees like that shown in Figure 4 can be constructed to provide the risk manager and/or regulator with information on the source of the risks and benefits associated with different control options. For example, various biosolids process-specific parameters, such as storage time or dilution within soil, can be varied and could impact the initial node decision of Figure 4. Wastewater treatment could be increased to counteract high shedding rates, which impacts the second decision node. Finally, educational programs for parents or those in charge of communal settings such as daycare centers, could be implemented impacting the third decision node. In addition to estimates on risks and benefits, specific sensitivity information on the decision to use a given control option can be obtained by examining the affects of varying different parameters in the model. For example, simulations could be conducted to provide information on the impact of changing the biosolids storage time for various levels of shedding and secondary transmission.

The model presented in this manuscript is deterministic; i.e., there is an assumption that the infection processes at the population level are completely determined by the causal factors incorporated into the model. This model structure is appropriate for studying large-scale outbreaks as well as general trans-

mission phenomena and similar models have been used in repeatedly (Brookhart et al. 2002, Eisenberg et al. 1996). There are numerous processes that are not accounted for in the deterministic model structure, some known but complex, such as climate and behavior, and others poorly understood, such as a variety of potentially important environmental factors. These processes result in stochastic fluctuations. The underlying assumption when using a deterministic model is that the disease states are represented by a large population so that any stochastic fluctuation is small compared to the overall trajectory of the model output. Stochastic phenomena, however, become important when one is interested in either modeling the risk of sporadic infections or in characterizing how heterogeneous factors affect risk. In both cases, states are often represented by small population groups and thus are more affected by stochastic processes. An example of the importance of heterogeneity is the distinction between the more intimate setting within a household compared to outside the household. This can be addressed by modeling both within-household transmission rates as distinct from between-household transmission rates using a stochastic model (Eisenberg et al. 2003-a). Since the purpose of this study was to introduce a framework of disease transmission that would provide general guidance on factors that might drive risk in a large population and in an endemic setting, the deterministic model was adequate. More specific risk assessment questions may indeed warrant a stochastic modeling approach.

Conclusions

One particular advance in the area of microbial risk assessment is the development of health risk models that explicitly account for the different transmission pathways that can result in human infection. The inclusion of these transmission pathways provided insight that would not be predicted by a chemical-based model. For example, the simulation results presented in this manuscript suggest that intermediate rates of shedding may result in a higher attributable risk than high rates of shedding. This is due to the fact that high rates of shedding result in high levels of pathogens in the environment, which in turn can result in pathogens circulating in the community at a high rate. Infection is spread throughout the community from person-to-person or person-to-environment-to-person. Eliminating any particular environmental pathway, therefore, will have little effect on the overall level of disease. By contrast, the chemical model would simply conclude that higher levels of shedding result in high levels of pathogens, and therefore higher levels of risk.

Risk assessment models can also provide information on data gaps and data needs. These data needs are crucial in the ability to estimate illness rate within a community or establish the adequacy of existing standards. Surveillance data for pathogens at various points in the environmental transport process, from a contamination site to an exposure site, provides information that can be used to improve the risk estimates provided by the model. Other types of environmental data useful when analyzing different scenarios are measurements of die-off both from natural and treatment factors. It is additionally valuable to have data that provides information on the functional relationship between these die-off factors and various environmental factors such as temperature and humidity. Some of the necessary health effects data are available to some degree in the literature. More detailed data on household-level transmission and the immune process would provide the needed information to incorporate additional structure into the model. In particular, a better understanding of how the different pathogen strains interact in developing immunity would be immensely useful.

To aid in interpretation of the simulations, results were presented in this paper through a structured decision tree of high and low attributable risk estimates for different environmental conditions. Describing the model output in the form of a decision tree can be a useful tool for the regulator by allowing the identification of factors that are the most important determinants of high and low risk conditions. In this form, the decision tree provides an assessment of the sensitivity of parameter values to risk levels. Although not formally done in this project, an uncertainty analysis could inform the regulator as to what additional research and data are needed to improve a given regulatory decision. This same decision tree can also be a useful tool at the operational level for outlining the health benefits associated with improving different components of the biosolids treatment process, for example, to determine the pathogen attenuation required to attain a low-risk condition.

Given the increasing interest and ongoing controversy in the beneficial uses of biosolids and the rapid growth in the public use of those biosolids, microbial risk assessment techniques are needed for application to biosolids regulatory and operational decision-making. A detailed and realistic risk assessment activity would require a comprehensive definition of relevant biosolids application scenarios and definition of relevant risk measures, and would provide the bridge between the model development and demonstra-

tion discussed in this report and an actual risk assessment. Scenarios that will be the most useful will be those that are framed as a comparative analysis. For example, one potentially interesting comparison is between occupational exposures that are, in general, associated with higher levels of exposure, longer exposure times, and fewer individuals exposed, and consumer exposures that are, in general, associated with lower levels of exposure, shorter exposure times, and larger number of individuals exposed. Every region in the country will have different exposure scenarios that are relevant to the particular conditions in which the biosolids are produced and used. For example, some may be more concerned with children exposed to biosolids applied near housing tracts while others may be more concerned with the potential of water runoff to contaminate sources of recreational or drinking water. In this paper, we describe a risk assessment methodology that has combined and adapted these advances to address human health risks from the exposure to pathogens within biosolids. With additional data collection and adaptation of appropriate exposure modeling methods, the risk assessment methodology presented has the potential to be applied at both the regulatory level, to review and revise the pathogen regulations for the Part 503 rule, and the operational level, to quantify differences in potential health impacts, or to evaluate environmental conditions and biosolids treatment processes.

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Pathogens in Biosolids - Microbiological Risk Assessment

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Nomenclature and Terms

- ID_{50} = dose of pathogen which when given to each and every member of a population, infects half of the members of that population.
- $AM_{treated}$ and AM_{raw} = the arithmetic mean pathogen levels in the treated and raw materials, respectively.
- Π_{surv} = the proportion of pathogens surviving a treatment process as determined by a single experiment.
- AM_{surv} = arithmetic mean of the proportions (Π_{surv}) of pathogens surviving the treatment process.
- MAD = mesophilic anaerobic digestion.

Introduction to Microbiological Risk Assessment for Pathogens in Biosolids

In the United Kingdom (UK), some 520,000 tonnes dry solids (tds) of sewage sludge (1996/97) are applied to agricultural land annually (WRc 1998). It is estimated that 119,025 tonnes of vegetable crops are produced annually in the UK from land to which treated sewage sludge has been applied (UKWIR 2003). This represents the total annual vegetable intake of 1,150,000 persons in the UK (~2% of the UK population). Microbiological risk assessment (MRA) approaches have been developed recently for modeling the exposures and risks to humans from pathogens potentially present on root crops, which have been grown on land to which treated sewage sludge was applied according to the UK guidance. The development of these models was funded and guided by UK Water Industry Research (UKWIR), the UK Environment Agency, and the Department of Environment, Food and Rural Affairs (Defra) under the management of UKWIR. The objective of this paper is to summarize the approaches and findings. The methods and results are described in full in UKWIR (2003).

The working assumption is that soil on vegetable crops at point of harvest may contain pathogens present in the treated sewage sludge. Root crops contain higher proportions of soil at point of harvest than leafy vegetables and may also be consumed uncooked (e.g. carrots). Root crops therefore present a worst case and for the purpose of risk assessment are modelled here. Human consumption data are available for root crops (Defra 2000) and are used to calculate pathogen exposures. Data are lacking on the proportion of root crops which are consumed raw, and also on the degree of processing and washing of vegetables in the kitchen prior to consumption. Worst-case assumptions are therefore used. The MRAs in UKWIR (2003) cover seven pathogens, namely salmonellas, *Listeria monocytogenes*, campylobacters, *Escherichia coli* O157, *Cryptospordium parvum*, *Giardia*, and enteroviruses. Here, MRAs are discusses for three of the bacterial pathogens: salmonellas; *Listeria monocytogenes*; and *E. coli* O157. In addition, the MRA developed for bovine spongiform encephalopathy (BSE) in sewage sludge in the UK is included (Gale and Stanfield 2001).

The UK guidance for land disposal of sewage sludge is set out in The Safe Sludge Matrix (www.adas.co.uk/matrix) and specifies both treatment of the sewage sludge prior to application to land

and also a harvest interval between application of the sludge and the harvesting of crops. In the case of vegetable crops (which are not eaten raw), the harvest interval is 12 months. For ready to eat crops, such as salad crops and carrots, this is extended to 30 months.

Environmental risk assessments are based on the source, pathway, receptor approach (Gale 2001a). "Source" represents the loading of pathogen in the raw sewage and "receptor" represents those humans who consume root crops grown on land to which treated sewage sludge was applied. The "pathway" sets out the route(s) by which the receptors are exposed to infectivity by the source. These are best modelled as event trees. An event tree defining the pathway by which salmonellas in raw sewage sludge are transmitted to root crops is shown in Figure 1. Central to quantifying the exposures are the pathway barriers. These include sewage sludge treatment (e.g. mesophilic anaerobic digestion or MAD), decay of the pathogens in the soil, and dilution of the pathogens after tilling of the treated sewage sludge into the soil.

The pathogen exposure to humans (the receptors) through consumption of raw root crops is translated into a risk of infection (or illness in the cases of *E. coli* O157) using the available dose-response data. Three dose-response models for salmonellas in humans are presented in Figure 2. It should be noted that the ID_{50} is the dose which when given to each and every member of a population, infects half of that population. It is not the "minimum infective dose". The ID_{50}s for infection (see lines (a) and (b) in Figure 2) are in the region of 10,000 to 100,000 cells. This does not mean that lower doses present zero risk; in fact according to the Beta-Poisson dose-response models fitted in Figure 2, even single salmonella cells have small but finite risks of initiating infection. For this reason, quantitative MRAs are appropriate even when the pathogen exposures are so low that most receptors only ingest a single pathogen cell (or particle).

Variation in Pathogen Levels on Root Groups After Land Application of Treated Sewage Sludge: Use of the Arithmetic Mean Pathogen Exposure in MRA

The arithmetic mean pathogen level in a medium (e.g. raw sewage (Gale 2003a), drinking water (Gale 2001a) or recreational water (Gale 2001b)) represents the total loading of pathogen in that medium. It gives no information on how those pathogens are dispersed within the medium. Typically pathogens are not evenly dispersed, but tend to be "clustered" both in space and time. Despite this, the arithmetic mean pathogen exposure to an individual receptor through a medium may be used directly in the dose-response

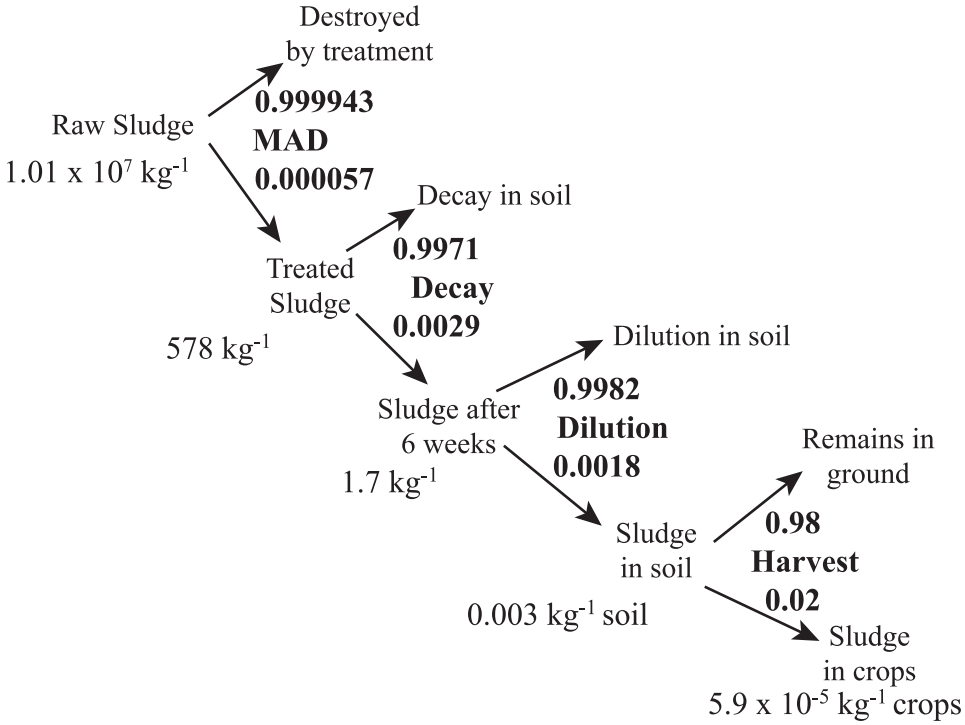

Figure 1. Event tree for transmission of salmonellas in raw sewage sludge to root crops. Adapted from UKWIR (2003).

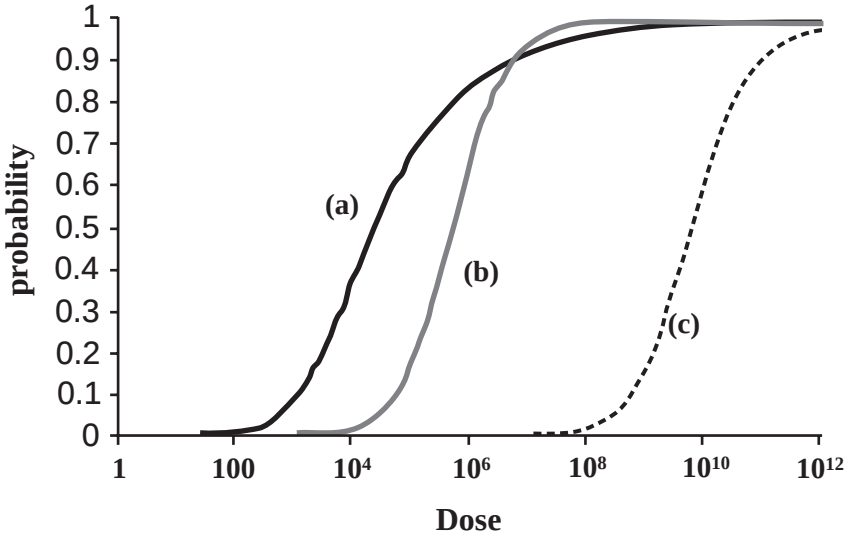

Figure 2. Dose-response curves for salmonella infection (a,b) and illness (c); Beta-Poisson models; a) non-typhi salmonellas, FAO/WHO (2000) (α = 0.3136; β = 3,008); b) *Salmonella meleagridis*, Teunis et al. (1999) (α = 0.89; β = 440,000); and c) *Salmonella pullorum*, Coleman and Marks (2000) (α = 0.70; β = 3.5 x 10^9). Taken from UKWIR (2003).

model to predict the risk of infection to that individual. This was shown mathematically by Haas (1996) and later demonstrated by Monte Carlo simulation to hold for *Cryptosporidium* oocysts in drinking water during a waterborne outbreak (Gale and Stanfield 2000). For highly infectious pathogens (e.g. rotavirus), however, this simple approach does not hold, and using the arithmetic mean actually overestimates the number of infected individuals by about threefold in the case of drinking water (Gale 2001a). This is because the arithmetic mean assumes complete dispersion of the pathogens within the medium and hence overestimates the number of individuals within the population who are actually exposed to pathogens (and subsequently infected). Furthermore, in situations where the pathogen exposure to an individual through a medium is high relative to the ID_{50} for that pathogen (Gale 2003a; 2003b), the overestimation could be considerable. The highest loading of *E. coli* O157 in lamb faeces was reported at >10^6/g (Strachan et al. 2001). Consider 1 g of that lamb's faeces and 10^6 persons potentially exposed, e.g. through walking in the countryside. Confining the 1 gram of faeces to one person would result in just that one person being exposed and infected. Dispersing the 1 gram of feces across 10^6 persons, such that each and every person was exposed to 1 cell of *E. coli* O157, would result in over 10,000 infections in the population (see Table 1 of Gale 2003a). Spatial heterogeneity and defining pathogen exposures to individual receptors is clearly important in MRA in such cases.

It is concluded that: 1. Using the arithmetic mean directly in the dose response model is worst-case; 2. Dispersion of pathogens increases the risk; while 3) Concentration (or "clustering") of pathogens minimizes the risk by confining exposure to just a few individuals.

To determine by how much simply using the arithmetic mean could overestimate the risk of infection from salmonellas in sewage sludge, Gale (2003a) developed a Monte Carlo simulation to model the counts of salmonellas on 1 kg batches of root crops at point of harvest. The results are presented in Table 1. To achieve relatively high salmonella levels, the model allowed for 2-log reductions of salmonellas by both sludge treatment and decay on the soil. According to the simulation, the majority of 1 kg root crop batches (99%) contained 0 salmonellas and 0.57% contained just a single salmonella cell. However, a very small proportion contained higher counts. Thus, 0.1% of 1 kg batches of root crops at point of harvest contained six or more salmonellas. The maximum in the 100,000 exposures drawn by Monte Carlo simulation was 706 salmonellas kg^{-1}.

The risk of salmonella infection to humans consuming raw (uncooked) 1 kg portions as calculated using each of those 100,000 exposures drawn form the log-Normal distribution (and the salmonella dose-re-

Table 1. Results of Prototype Monte Carlo Simulation*: Poisson-log-Normal Distribution for Salmonella Counts on 1 kg Portions of Root Crop at Point of Harvest. Taken from Gale (2003a) and Assuming 2-log Destruction by Sludge Treatment and 2-log Decay in Soil.

Salmonella Count Bin	Bin %	Cumulative %
0	99.083	99.083
1	0.573	99.656
2	0.131	99.787
3 to 5	0.115	99.902
6 to 10	0.045	99.947
11 to 50	0.039	99.986
>50	0.014	100

*arithmetic mean of 100,000 simulated exposures was 0.046 salmonellas kg^{-1}.

sponse curve in Figure 2 (line (a)) was very similar to that calculated by using the arithmetic mean (0.046 salmonellas kg^{-1}) directly in the dose-response curve. Thus, using the arithmetic mean directly in the dose-response curve, predicted 0.483 infections per 100,000 persons. Using the simulated log-Normal distribution predicted a slightly lower risk with 0.468 infections per 100,000 persons. These estimates are very similar supporting the use of the arithmetic mean pathogen exposure for MRA purposes.

Risk Assessment Approaches to Calculating the Arithmetic Mean Pathogen Concentration in Raw Sludge

The preliminary objective of the risk assessment is to estimate the arithmetic mean pathogen level in the raw sewage sludge (AM_{raw}). In the case of salmonellas and *Listeria monocytogenes*, monitoring data are available for levels of pathogens in raw sewage (Watkins & Sleath 1981). These data may be used directly in MRA by constructing an event tree to model the partitioning of the pathogens from the raw sewage into the raw sludge (Gale 2003a). This in effect represents what happens at a sewage treatment works and is illustrated in Figure 3 for salmonellas. According to the event tree, each 1 litre of raw sewage contributes 2,713 salmonellas to the raw sludge. Since 100 litres of raw sewage are required to make each litre (wet weight) of sludge (CIWEM 1996), the level of salmonellas in the wet sludge is 271,300 /litre. The dry solid content of wet sludge is 2.7% (w/w). Therefore, 1 kg (dry solids) contains 1.01×10^7 salmonellas. This is equivalent to 1.01×10^{10} salmonellas/tds.

There are no monitoring data for BSE or *E. coli* O157 in raw sewage or sewage sludge on which to base a quantitative MRA. The main source of BSE agent and *E. coli* O157 in raw sewage is from abattoirs. In the case of BSE, small amounts of brain and spinal cord potentially enter the sewer after butchering of the carcass (Gale and Stanfield 2001), while sheep and cattle feces discharged at the abattoir contribute *E. coli* O157 (in addition to other fecal pathogens). Quantitative MRAs for these two agents are possible on the basis that only the arithmetic mean level in sewage sludge is required. Central to calculation of the arithmetic mean is the fact that in total 967,000 tonnes dry solids (tds) of sewage sludge are produced annually in England and Wales (WRc 1998). The loading of BSE agent to the sewer in England/Wales, assuming 1% of bovine brain and spinal cord entered the sewer, was estimated at 378,000 bovine oral ID_{50}s $year^{-1}$ (Gale and Stanfield 2001) giving an arithmetic mean of 0.39 bovine oral ID_{50}s tds^{-1} of raw sewage sludge.

The loading of *E. coli* O157 to the sewer from the annual slaughter of 3.13 million cattle and 15.86 million sheep at abattoirs in England and Wales assuming, on the basis of expert advice, that 5% of fecal material in slaughtered animals enters the sewer, is 2.9×10^{13} cfu $year^{-1}$. From the event tree for salmonellas (Figure 3), 82.9% of *E. coli* O157 (i.e. 2.4×10^{13} cfu /year) would partition into the 967,000 tds of raw sewage sludge produced annually in England/Wales giving a concentration of 2.5×10^7 cfu tds^{-1} of raw sewage sludge. This is considerably lower than the 1.01×10^{10} tds^{-1} predicted for salmonellas in raw sludge (see Figure 3).

Using the Arithmetic Mean Pathogen Removal in MRA

The efficiency of removal of pathogens by treatment processes such as drinking water treatment (Gale and Stanfield 2000; Gale et al. 2002) or sewage sludge treatment (Gale 2003a) varies both "between batch" and "within batch" reflecting operational conditions. The net removal of pathogen is represented by the arithmetic mean removal and is calculated as the arithmetic mean (AM_{surv}) of the proportion of pathogens

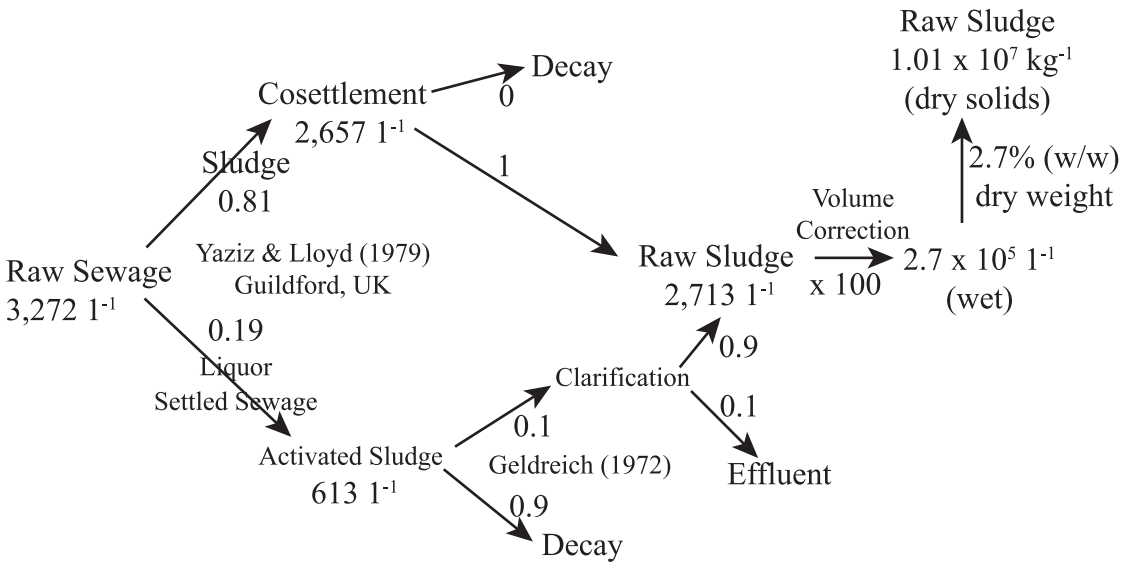

Figure 3. Event tree to model the partitioning of salomellas in raw sewage into raw sewage sludge. Taken from UKWIR (2003).

which survive, or breakthrough, the treatment process. Thus, if a single experiment is conducted to measure the pathogen density in a medium (e.g. sewage sludge or drinking water) before treatment, X_{raw}, and after treatment, $X_{treated}$, then the proportion of pathogens surviving the treatment process is calculated as $_{surv} = X_{treated} / X_{raw}$. For n such experiments:

$$AM_{surv} = \frac{1}{n}\sum_{i=1}^{n}(\pi_{surv})_i \qquad (1)$$

It may be shown mathematically for log-Normally distributed variables (Gale, unpublished) that the arithmetic mean pathogen level in the treated product ($AM_{treated}$) is the product of the arithmetic mean pathogen level in the raw material and the arithmetic mean removal of pathogen by treatment. Thus:

$$AM_{treated} = AM_{raw} \times AM_{surv} \qquad (2)$$

This is intuitively the same as saying the total number of pathogens in the treated product equals the total number in the starting material minus the total number taken out by treatment. Equation 2 may be extended to allow for pathogen decay and dilution in the soil to calculate the arithmetic mean pathogen loading in the soil (AM_{soil}) at point of harvest of the crops. Thus:

$$AM_{soil} = AM_{raw} \times AM_{surv} \times AM_{decay} \times AM_{dilution} \qquad (3)$$

Where AM_{decay} is the mean pathogen decay in soil and $AM_{dilution}$ is the mean dilution factor of the sewage sludge in the soil.

This approach greatly simplifies quantitative MRA and eliminates the need for using complex and time-consuming Monte Carlo simulations to model the variation in pathogen exposures to individual humans. Furthermore, the approach focuses the uncertainty into the individual terms and simplifies sensitivity analysis. Thus, for example, a 10-fold increase in the net pathogen removal by treatment will decrease the pathogen level in the treated product (and hence the risks) by 10-fold. This is straightforward to understand; the problem is determining experimentally, in the face of potentially huge "between batch" and "within-batch" variation at operational scale, whether the arithmetic mean removal by treatment has been improved by 10-fold (Gale 2001b).

Arithmetic mean pathogen removals (presented as logarithms) by MAD are summarised in Table 2 for the four pathogens considered here, together with soil decay parameters. Dilution of the sludge in the soil is based on an application rate of 6.57 tds/ha/year. In terms of the event tree (Figure 1), dilution represents

Table 2. Summary of Log Destructions by MAD and Log Decays in Soil (as used in UKWIR 2003)

Pathogen	Log_{10} destruction by MAD		Log_{10} destroy in the soil	
	Operational Efficiency 100%	Operational Efficiency 99%	Experimental range (time)	Extrapolated to 6 weeks (42 d)
Salmonella	4.24[a]	1.997	2.11[b] (5 weeks)	2.53
Listeria monocytogenes	2.23[a]	1.800	3.0[c] (30 days)	4.2
E. coli O157	3.8[a]	1.993	4.5[d] (50 days)	
BSE[e]	0	0	0	

[a]UKWIR (2002)
[b]Watkins and Sleath (1981)
[c]Data from ADAS
[d]Bolton et al. (1999)
[e]Gale and Stanfield (2001)

the probability ($AM_{dilution}$) of a root crop colliding with a sludge particle at point of harvest, compared to the much greater probability of colliding with a soil particle (Gale and Stanfield 2001).

Predicting Risks and Exposures to Humans through Consumption of Root Crops

Using the event tree in Figure 3, the arithmetic mean level of salmonellas in the raw sewage sludge (AM_{raw}) is estimated at 1.01 x 10^7 cfu kg^{-1} (see UKWIR (2003) for complete description). Feeding this value into the pathway event tree (Figure 1), predicts an arithmetic mean salmonella level in soil (6 weeks after sludge application) of 0.003 cfu kg^{-1} by Equation 3. Thus:

$$AM_{soil} = 1.01 \times 10^7\ kg^{-1} \times 0.000057 \times 0.0029 \times 0.0018 = 0.003\ kg^{-1}$$

Data from UK crop growers show that root crops contain up to 2% (w/w) soil at point of harvest (Gale 2003a). Therefore each 1 kg of root crops at point of harvest contains 0.02 kg of soil/sludge. This is the origin of the 0.02 in the event tree (Figure 1), and the arithmetic mean level of salmonellas on root crops at point of harvest is 5.9 x 10^{-5} cfu kg^{-1}. For the purpose of MRA, it is assumed that washing of the root crops prior to consumption removes 90% of the soil. This could either be visualised as 90% of soil being removed from all roots crops, alternatively as 90% of root crops being thoroughly cleaned and 10% being consumed unwashed. After washing therefore, the arithmetic mean salmonella level on root crops is 5.9 x 10-^{6} cfu kg^{-1}.

According to data from Defra (2000), each person in the UK ingests 1,986 g of vegetable and vegetable products per week. Thus, the total vegetable consumption in the UK is 284 g $person^{-1}$ day^{-1}. Of this, 70 g $person^{-1}$ day^{-1} comprise "other fresh vegetables" including root crops such as carrots and radishes which are eaten raw some of the time. On the basis that 50% of such crops are likely to be cooked, the risk assessment is based on each person ingesting 35 g day^{-1} of uncooked root crops. This is 12.77 kg/person/year, which contributes an arithmetic mean salmonella exposure of 7.6 x 10^{-5} cfu/person/year (Table 3). Using the FAO/WHO dose-response relationship (line (a) in Figure 2), this exposure translates into an individual risk of salmonella infection of 7.9 x 10^{-9} /person/year (Table 3). Such a risk is not only remote, but also worst case in the sense that the decay period in the soil is limited to just 6 weeks (Figure 1). According to the UK Safe Sludge Matrix, the time interval between application of MAD-treated sludge and harvesting of ready-to-eat crops (e.g. carrots) should be 30 months. Linear extrapolation of the salmonella decay in soil to a period of 12 months predicted a salmonella level of 6.4 x 10^{-23} kg^{-1} of root crops (Gale 2003a). Exposures and risks from *Listeria monocytogenes*, *E. coli* O157 and BSE are also set out in Table 3. It should be noted that the BSE risk assessment is very worst case in allowing for no destruction by sewage sludge treatment and no decay in the soil (see Gale and Stanfield 2001). In addition, the BSE exposure presented in Table 3 assumes that cooking has no effect on the BSE prion by taking into account all 284 g of total vegetables consumed per person per day (Defra 2000).

Predicting the Number of Infections in the UK Population

When the USEPA promulgated the Surface Water Treatment Rule for treatment of drinking water, its goal was to ensure that the population consuming water would not be subject to a risk of greater than one infection of giardiasis per 10,000 persons per year (Regli *et al*. 1991). In effect, therefore, the acceptable risk to the individual consumer of infection by *Giardia* through drinking water is 10^{-4}/person/year. In a population of 60 million people (e.g., in the UK) this would translate into 6,000 *Giardia* infections per year through

Table 3. Summary of Predicted Pathogen Exposures and Risks to Humans from Consumption of Root Crops Grown on Soil to which MAD-treated Sewage Sludge was Applied. MAD-removal and Soil Decay Rates Presented in Table 2.

Pathogen	Decay time on soil	Exposure (/person /year)	Risk of infection (/person/year)	Number of infections (persons/year)	Dose response data
Salmonella	42 days	7.6×10^{-5} cfu	7.9×10^{-9}	0.009	FAO/WHO (2000) Line (a) in Figure 2
Listeria monocytogenes	42 days	0.0024 cfu	1.15×10^{-8}	0.013	10401 strain mice; Haas *et al*. (1999)
E. coli O157	50 days	5.6×10^{-9} cfu	[a]5.9×10^{-11}	[a]0.000068	[a]Crockett *et al* (1996)
BSE[b]	--	1.42×10^{-8} human oral ID_{50}	0.98×10^{-8}	0.011	Gale (1998)

[a]Illness

[b]Recalculated from Gale and Stanfield (2001) assuming 1% of cattle brain and spinal cord enters sewer, total crop consumption is 284 g/person/day and washing removes 90% of soil.

drinking water alone. There are also other enteric waterborne pathogens to consider, and also other routes of transmission to humans, such as direct contact with animals (and animal faeces), food, and recreational activities. Indeed, risk assessments should address the "big picture" by considering all routes, rather than single routes in isolation (Gale 2002). This raises the question of what is an acceptable risk of infection to humans in the UK through consumption of root crops, which have been grown on land to which treated sewage sludge was applied according to The Safe Sludge Matrix. To address this, the total number of persons infected annually in the UK (UKWIR 2003) was calculated as the product of the annual, individual risk from consumption of root crops and the number of persons in the UK exposed to that risk from consuming root crops grown on sludge-treated land.

For the purpose of MRA, it was estimated that 119,025 tonnes per year of vegetable crops are produced in the UK on land to which sewage sludge has been applied (UKWIR 2003). On the basis that the total vegetable consumption in the UK is 284 g person^{-1} day^{-1} (i.e. 0.1035 tonnes person^{-1} year^{-1}), it may be calculated that 1.15×10^6 persons in the UK consume vegetable crops (all year) grown on land treated with sewage sludge. This figure is used in the risk assessment. Thus, the number of salmonella infections from consumption of crops grown on sewage sludge treated fields is calculated as 6.9×10^{-9} infections/person/year x 1.15×10^6 persons = 0.009 infections/year (Table 3).

The 119,025 tonnes of crops grown annually on sludge-treated fields in the UK will undoubtedly be distributed unevenly across the UK population. However, this does not affect the overall number of infections predicted by MRA because of the linear nature of the dose-response relationship at low doses. Thus, with regard to vegetables grown on sludge-treated fields, whether 1.15×10^6 persons ingest 284 g/person/day for all 365 days of the year, or whether all 60×10^6 person in the UK ingest 284 g/person/day for just 6.99 days of each year, the total consumption is still 119,025 tonnes of vegetables. The risk to the population as a whole is the same (0.009 salmonella infections/year) for both scenarios (calculation not shown).

It is concluded that the predicted numbers of infections in the UK from consumption of root crops grown on land to which treated sewage sludge has been applied are remote (Table 3). The highest predicted risk is for *Listeria monocytogenes*, with 1 infection in the UK every 77 years. The predicted risks from *E. coli* O157 are more remote still with 1 illness predicted in the UK every 14,000 years.

Uncertainty In The Uncertainty

"One problem with quoting quantitative predicted risks is that the degree of uncertainty is quickly forgotten" (P. Gale cited in Macgill *et al*. (2001)). A natural extension of calculating the arithmetic means for the source, pathway and receptor terms, and indeed for the risk of infection, is to calculate the uncertainty, and quote the 95% confidence intervals. Indeed, it would be greatly reassuring for a risk assessor to state with

95% certainty that the expected number of infections is no less than x cases, but no greater than y cases per year. It could be argued, however, that there are not sufficient data and understanding at present to determine the uncertainty. Sources of uncertainty reflects the following:

1. Missing "rare but all important" high count samples in the source term data (Gale and Stanfield 2000).

2. Paucity of operational data on the efficiency of the sludge treatment processes with particular reference to "bad-days and by-pass" (Gale 2002).

3. Lack of knowledge on the nature of the decay of pathogens in the soil with respect to extrapolation of decay data to the 12 or 30 month harvest intervals specified by The Safe Sludge Matrix. Thus, for extrapolation, it is assumed that all cells of a certain pathogen have exactly the same probability of death in the soil giving a linear reduction in the log-transformed counts over time. The presence of a more resistant sub-population would give non-linear decay.

4. Lack of knowledge on the nature of dose-response relationship at low pathogen doses, e.g. cooperative or independent action (Gale 2003b), acquired protective immunity within the population and susceptible sub-populations.

To estimate the uncertainty, assumptions have to be made, e.g. in the nature of a statistical distribution, and with assumptions comes more uncertainty. Therefore the problem with quoting the uncertainty, is that the uncertainty in the uncertainty is not, as yet, fully understood.

Uncertainty In Removal By Treatment: Good Days And Bad Days

Of critical importance to the risk assessment is the uncertainty in the estimation of AM_{surv}. The design of experiments to quantify AM_{surv} is complicated by the potentially large "between batch" and "within batch" variation, particularly at operational scale. This is illustrated in Figure 4 by plotting the proportions of enterovirus surviving sewage sludge digestion at operational scale. Each point represents a monthly experiment. AM_{surv} is 0.0916 (i.e. 9.16% of enteroviruses survive overall) which is in the order of a 1-log destruction. Some experiments showed high destructions in the region of 2 - >3 logs (good days), while other experiments revealed poor destruction of <1-log (bad days). The problem is that the "good days do not compensate for the bad days" (Gale 2001b) and AM_{surv} is weighted towards the "bad days". Furthermore, the majority of single experiments will underestimate AM_{surv}, and in some cases by a considerable amount. In this respect, an understanding of the variation is critical for interpreting experimental data. Thus, according to the log-Normal distribution fitted to the data in Figure 4, some 29% (shaded area) of single experiments will underestimate the AM_{surv} (and hence the risk of infection in the population) by more than 10-fold. Thus 1 in 3 experiments will give a picture of sewage sludge treatment which is at least 10-fold too optimistic. For the Beta distribution (which is also fitted to the data in Figure 4), 1 in 5 (i.e. 20%) experiments underestimate AM_{surv} by more than 10-fold. This demonstrates the difficulty in estimating experimentally the net pathogen destruction by sludge treatment.

What If? Scenarios: Operational Efficiency Of Sewage Sludge Treatment "By-Pass"

The predicted risks in Table 3 assume that the net pathogen destruction at operational scale is the same as that determined at laboratory scale (UKWIR 2002) and in effect that the operational efficiency is 100%. This raises the question of, "What if the sludge treatment process is only 99% efficient?". This is sometimes described as "by-pass" (Gale 2002). By-pass in its simplest form is when the final treated product is "contaminated" by the raw material (Bendixen 1999). The effect of by-pass on AM_{surv} can be huge (Gale 2003a) and may be calculated using Equation 1. Thus, MAD operated at 100% efficiency destroys 4.24-logs of salmonella destruction (Table 2). However, if MAD is operated at only 99% efficiency, such that 1% is completely untreated, then the net destruction is 1.997-logs (Table 2). In Table 4, the number of infections are calculated for 99% operational efficiency. For the three bacterial pathogens considered, a 1% by-pass of MAD at operational scale has little impact on public health, even allowing for just 42 days decay on the soil. Thus, the number of salmonella infections increases by 175-fold from 0.009/year to 1.6/year with 1% by-pass (Table 4). Allowing for a 12 month harvest interval (The Safe Sludge Matrix specifies 30 month) would reduce the number of salmonella infections to $<10^{-14}$ /year, which is a remote risk.

Conclusions

- Models are developed to predict the number of infections annually in the UK from consumption of root crops grown on land to which MAD-treated sewage sludge has been applied. The pathogens studied are *E. coli* O157, *Listeria monocytogenes* and salmonellas.

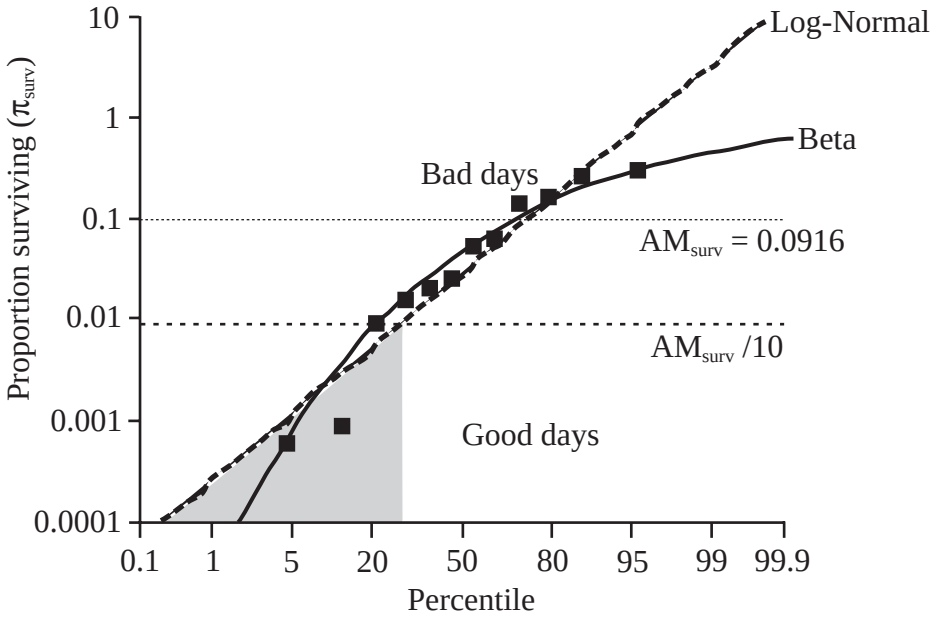

Figure 4: Distribution of proportions (π_{surv}) of enteroviruses surviving anaerobic digestion (data calculated from Soares et al. 1994). Log-normal (a) μ_{surv} = -1.542; σ_{surv} = 0.894 (log_{10}) and Beta (b) α = 0.538; β = 5.341 distributions fitted to data. Adapted from Gale (2003a). Shaded area represents 29%.

Table 4. What if Operational Efficiency of MAD is Only 99%? Net MAD Removals at 99% Operational Efficiency are Presented in Table 2

Pathogen	Decay time on soil	Number of infections in UK (persons/year)	
		Operation Efficiency of MAD	
		100% (Table 3)	99%
Salmonellas	42 days	0.009	1.6
Listeria monocytogenes	42 days	0.013	0.036
E. coli O157	50 days	0.000068[a]	0.004 [a]

[a]Number of illnesses

- Even with just a 6 week interval between the application of the MAD-treated sludge and the harvesting of the crops, the risks are remote with one salmonella infections predicted in the UK population every 111 years.
- Operational efficiency of MAD is important and reducing efficiency to 99% increases the predicted number of salmonella infections to 1.6 per year in the UK population. Allowing for pathogen decay over the full 30 month harvest interval as specified by the UK Safe Sludge Matrix would eliminate the risks completely.
- A formal uncertainty analysis has not been undertaken because there would be uncertainty in the uncertainty, and processes such as pathogen decay in the soil over 12 and 30 month intervals are not fully understood at present. In general, uncertainty has been overcome by using worst-case assumptions where data are not available.

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Relevance of Microbial Risk Assessment for Foodborne Pathogens to Water Safety

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Introduction

Microbial risk assessment for foodborne pathogens evolved from the chemical risk assessment paradigm and early research related to water safety concerns. In the United States, several extensive risk assessments have been conducted that offer an important body of experience. Exposure assessment and hazard characterization of waterborne and foodborne pathogens are reviewed in order to distinguish similarities and differences in approaches for dealing with these two microorganism classes. As the increasing number of incidents linked to produce consumption indicates, water and food safety concerns are often different manifestations of the same issue. It can thus be expected that microbial risk assessment for waterborne and foodborne pathogens will tend to follow similar frameworks, and, in some instances, might ultimately converge in assessments of the aggregate risk.

Notion of Waterborne and Foodborne Pathogens

Microbial concerns in water and food safety converge around pathogens that have a fecal-oral transmission cycle. The terms waterborne and foodborne essentially describe a mode of transmission rather than an inherent characteristic of a pathogen. Indeed, most pathogens can follow both paths as well as others, such as person-to-person contact.

In this context, the case of *Escherichia coli* O157:H7 is very illustrative. Based on the number of waterborne outbreaks reported in the United States from 1993 to 1998 (Barwick et al., 2000; Kramer et al., 1996; Levy et al., 1998), this organism ranks among the most important waterborne pathogens, second only to the parasites *Cryptosporidium parvum* and *Giardia* spp. (see Figure 1). As shown in Figure 2, *E. coli* O157:H7 outbreaks have been associated with a variety of vehicles including ground beef, coleslaw, drinking water, apple juice, recreational contact with water, contact with farm animals, and others (CDC, 1999a; CDC, 2000). The largest outbreak reported in 1999 was linked to drinking water available at a county fair, where more than one thousand persons were affected, eleven suffered hemolytic uremic syndrome, and two died (CDC, 1999b). However, all vehicles bear the potential to cause large outbreaks, and it is rather the number

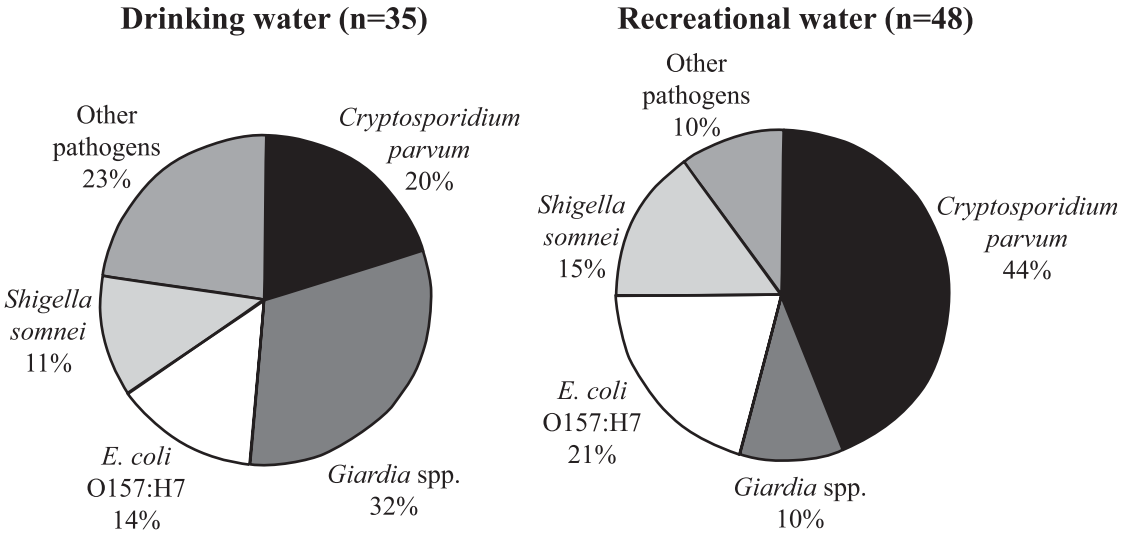

Figure 1. Pathogens Involved in Reported Waterborne Outbreaks with Known Infectious Etiology, United States 1993–1998 (Barwick et al., 2000; Kramer et al., 1996; Levy et al., 1998)

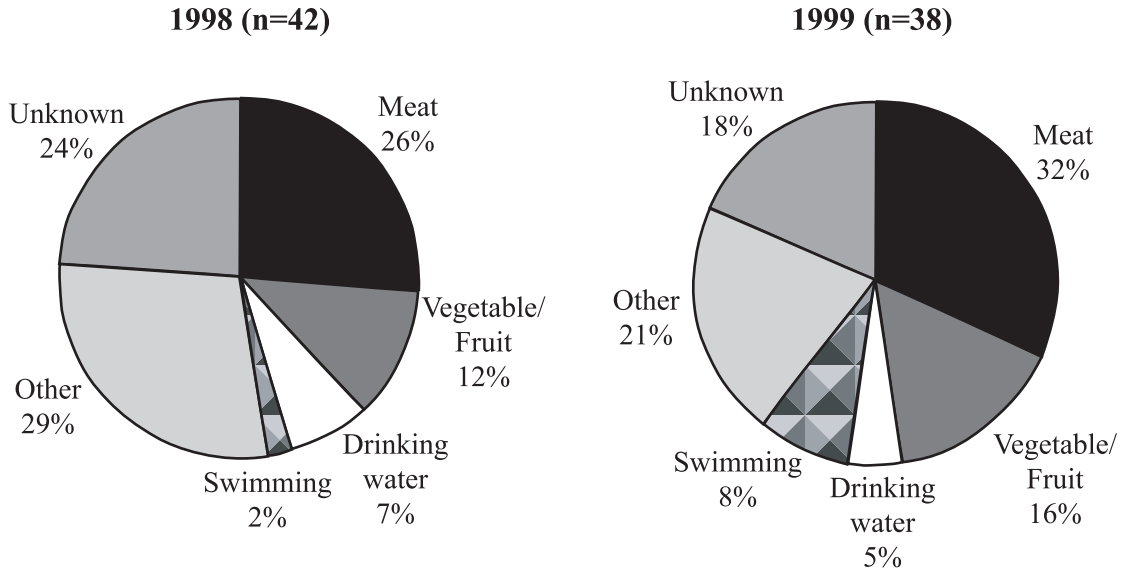

Figure 2. Vehicles Associated with *E. coli* O157:H7 Outbreaks Reported in the United States in 1998 and 1999 (CDC, 1999a; CDC, 2000)

of potentially exposed individuals that determines the magnitude of an outbreak. As for many other pathogens of interest to both water safety and food safety, *E. coli* O157:H7 transmission occurs along an environmental continuum linking animals to humans.

Water and food are merely two exposure scenarios over which the continuum exists. It is noted that *E. coli* O157:H7 provides a good example of one of the primary motivations for conducting microbial risk assessments. Not only is the total number of acute gastroenteritis cases of concern, but so is the severity in certain populations. In particular, there is an interest in quantifying the occurrence of specific complications (e.g., hemolytic uremic syndrome) in particular population subgroups (e.g., children).

Mead et al. (1999) estimated the burden of illness and death caused by bacterial, viral, and parasitic foodborne pathogens in the United States. Several foodborne pathogens considered in the estimate are likewise of water safety concern, e.g., *Shigella* spp. and *Cryptosporidium parvum*. An important component

of Mead's analysis is the adjustment of the number of reported cases by the estimated proportion of foodborne transmission. While the discussion focuses around the estimated 13.8 million cases of acute gastroenteritis and 2,700 deaths due to foodborne transmission, those estimates represent 36% of all gastroenteritis cases and 67% of all deaths, respectively. In other words, although foodborne transmission certainly has relevant consequences, other modes of exposure also play an important role. This observation highlights a crucial shortcoming of the dichotomization between waterborne and foodborne transmission. While the need to evaluate health consequences from different pathogens via a common pathway — that is, the assessment of the cumulative risk — is frequently recognized, the importance of estimating the health effects from a given pathogen across several exposure pathways — the assessment of the aggregate risk — is commonly overlooked. Given the fact that few pathogens actually dominate public health concerns, the assessment of the aggregate risk appears to be at least as essential as that of cumulative risk. However, the compartmentalization of responsibilities among various governmental agencies would seem to be an important impediment to their realization.

Differences and Similarities between Waterborne and Foodborne Pathogens Relevant to Microbial Risk Assessment

From a broad perspective, the distinction between waterborne and foodborne pathogens is functional. In terms of public health, it can be inconsequential and even potentially misleading. Nonetheless, the classification outlines some background differences and similarities that can help direct risk assessors' efforts. Those practical distinctions are outlined in Table 1, and are postulated to generate further discussion rather than provide definitive evidence.

Food safety efforts have mainly been directed at bacterial pathogens. Historically, this is because bacteria have been better characterized. Moreover, bacteria find — under given conditions — an adequate milieu for growth in food items. In contrast, water offers a limited potential for microbial growth, and thus pathogen survival becomes the essential attribute. Parasitic protozoa and ova seem to have an inherent advantage in this respect. Human enteric viruses are a food safety concern in produce, shellfish, and ready-to-eat

Table 1. Exposure Characteristics Inherent in Waterborne and Foodborne Transmission Modes

	Waterborne transmission mode	**Foodborne transmission mode**	**Shared by water- and foodborne transmission modes**
Pathogen class of primary concern	Parasitic protozoa and ova	Bacteria	Enteric viruses
Critical event along the shedding to exposure continuum	Pathogen survival	Pathogen growth	Contamination
Primary place of contamination	Watershed	Anywhere along continuum	
Exposure frequency	Single exposure to extended over time (weeks/months); possibly seasonal/ intermittent	Single meal	Averaging time less than one day
Exposure intensity (relative dose)	Low (High, for water treatment failure)	High	
Control options	Few, i.e. barriers to contamination source & water treatment	Several; at different stages of food production, harvesting, processing, preparation	
Responsibility	Water distributor	Theoretically, anyone along continuum; practically, processors	

foods as a result of fecal contamination. In this case, a food item functions as a vector in very much the same way that water does. The main conclusion that can be drawn from this general observation is that the key difference between risk assessment for waterborne pathogens and that for foodborne pathogens lies in the exposure process. Opportunities for fecal contamination seem to be better definable for water than food, but a limited number of risk abatement options may exist for the water medium. Also, exposure to waterborne pathogens generally consists of lower doses but persisting over a longer period of time.

From the moment of ingestion, differences between transmission modes essentially relate to the duration and conditions of the gastric passage. A water medium may greatly reduce the transit time. Although ingestion of food increases the passage duration and stimulates the secretion of gastric acids, specific meal composition can also protect the pathogen. It is difficult to substantiate the net effect of these circumstances. Clearly, once the gastric barrier has been overcome, pathogens exert their effects in the gut regardless of their original medium. An important note is that the ability of bacteria to withstand the effects of gastric acids can be linked to the growth conditions of the microorganism. For instance, cattle that are fed grain (which lowers the colonic pH) have up to 106-fold more acid-resistant *E. coli* than do cattle fed hay (Diez-Gonzalez et al., 1998). Regardless of the mode of transmission, this stresses the need to consider the interaction between the pathogen and its vehicle explicitly in a risk assessment.

Relevant Aspects of Microbial Risk Assessment for Foodborne Pathogens

Risk assessment for microbial hazards is an emerging tool for unraveling the factors that contribute to the relevance of foodborne diseases (Jaykus, 1996; Lammerding & Paoli, 1997). It can thus provide sound guidance in the development and implementation of food safety policies. The Codex Alimentarius Commission (CAC) of the United Nations' Food and Agriculture Organization and the World Health Organization is the international body that elaborates food safety principles and guidelines. With regard to microbial risk assessment, CAC proposes an approach based on four distinct steps: hazard identification, exposure assessment, hazard characterization, and risk characterization (CAC, 1999).

This four-step approach heavily draws on the influential report of the U.S. National Research Council (NRC) entitled "Risk Assessment in the Federal Government: Managing the Process" (NRC, 1983). This document, now known as the "Red Book", collected the body of risk assessment experience in the United States. Formal risk assessment was initiated as early as the 1930s in the U.S. with a focus on noxious agents in industry. Risks posed by ionizing radiation were assessed starting in the 1960s, and carcinogens in food in the 1970s. In the early 1970s, the Environmental Protection Agency and the Occupational Safety and Health Administration were established, and have become major proponents and users of health risk assessments within the U.S. government (Boroush et al., 1998; U.S.Congress/OTA, 1993). Given its genesis, it is not surprising that the focus of the document is on environmental concerns, in particular those posed by carcinogens. A direct inheritance from the industrial setting and consequent engineering leaning is the tendency to view complex situations as modular, mechanistic systems. Other disciplines, in particular toxicology and epidemiology, are seen more as sources of data than of methods. This historical perspective provides an important insight when it comes to evaluating the current practice of microbial risk assessment. It must also be pointed out that research in such a field was first initiated for waterborne pathogens (Rose et al., 1991; Haas et al., 1993). Nonetheless, comprehensive efforts in conducting microbial risk assessments at the U.S. federal level have been limited to foodborne pathogens. In the process, several methods and data originally developed for waterborne pathogens have been adapted for foodborne pathogens.

While CAC lays out general guidelines for conducting microbial risk assessment, a framework by the Risk Science Institute of the International Life Sciences Institute (ILSI/RSI) seems to provide more details (ILSI/RSI, 2000). The goal of a microbial risk assessment is the evaluation of the likelihood of health effects from exposure to a pathogen. The systematic definition of the goals, breadth, and focus of the risk assessment is the objective of hazard identification. In food safety, this practically translates into identifying food items, pathogens and health effects of concern. The interaction between pathogen, the environment, and the human population is the focus of the exposure assessment. Through pathogen characterization, determination of pathogen occurrence, and exposure analysis, an exposure profile should result. Hazard characterization intends to evaluate the microorganism's ability to cause the health effect, and is composed of host characterization, evaluation of health effects, and quantification of the dose-response relationship. A host-pathogen profile is its output. The final step of risk characterization combines the exposure and host-pathogen profiles into the likelihood of adverse human health effects.

As of June 2001, four major food safety risk assessments have been carried out or are close to completion in the United States. In 1998, the Food Safety Inspection Service of the U.S. Department of Agriculture

(USDA/FSIS), in collaboration with the Food and Drug Administration, completed a risk assessment for *Salmonella Enteritidis* in shell eggs and egg products (USDA/FSIS, 1998a). USDA/FSIS has also conducted a risk assessment for *E. coli* O157:H7 in ground beef (USDA/FSIS, 1998b). The Center for Food Safety and Applied Nutrition of the Food and Drug Agency (USDA/FSIS, 2003) has completed risk assessments for *Listeria monocytogenes* in selected ready-to-eat foods (in collaboration with USDA/FSIS), and for *Vibrio parahaemolyticus* in raw molluscan shellfish (FAO/WHO Consultation, 2002). With the exception of Canada, where similar works for *Salmonella*, *E. coli*, *Listeria*, and *Campylobacter* have been conducted, few countries have completed food safety risk assessments with the breadth of those listed here. A common thread of the four U.S. risk assessments is that the hazard identification is not extensive. In particular, systematic methods available from reliability engineering, such as Failure Mode and Effects Analysis (Kumamoto & Henley, 1996), have never been applied in food safety. The risk assessments conducted by FDA/CSFAN closely follow the four-tiered process proposed by the CAC, but such steps are not immediately evident in the studies done by USDA/FSIS. While the USDA/FSIS assessments would seem to mimic in details the farm-to-fork continuum, the works done by FDA/CSFAN put a greater emphasis on the events from retail on. Since both agencies acknowledge the significance of the farm-to-fork continuum in their food safety efforts, the differences in emphasis in their risk assessment efforts may reflect the agencies' differences in mandates.

Although not yet completed, the USDA/FSIS risk assessment for *E. coli* O157:H7 in ground beef (USDA/FSIS, 1998b) is a good illustration of the organization of food safety risk assessments and their potential relevance for water safety. Figure 3 reproduces the intended layout of that work. The exposure assessment is split into three modules. In the production module, the number of affected cattle that are slaughtered is calculated. The harvesting module establishes the level of bacterial contamination at the end of slaughter, and the processing module calculates the changes in the initial contamination level as the product goes through grinding, storage, and cooking. Two elements of this exposure assessment appear to be of intrinsic interest to water safety risk assessment. First, in contrast to water safety risk assessments that usually start from a contaminated water medium, there is an overt attempt to consider the farm (and field) conditions. This not only offers the opportunity to model the source of contamination explicitly, but also provides opportunity to weigh potential risk abatement options. The second point is that the methods of predictive microbiology used for modulating contamination levels can be applied to simulating pathogen dilution and survival (i.e., the two events of potential relevance in an exposure assessment of waterborne pathogens). As for the further components of the risk assessment, the public health module merges hazard characterization and risk characterization. While there is the intention to consider various health effects in different population subgroups, the draft document mainly discusses the choice of pertinent data from human feeding trials and of a dose-response model. It is concluded that *Shigella dysenteriae* data are the most appropriate surrogate for *E. coli* O157:H7, and that the beta-Poisson model best fit the *Shigella* data. Such an approach is common in food safety risk assessment, and has two inherent limitations. The first limitation is that some degree of uncertainty is associated with the choice of a surrogate. The second limitation is that hazard characterization is essentially reduced to a dose-response assessment. Because of this second limitation as well as the

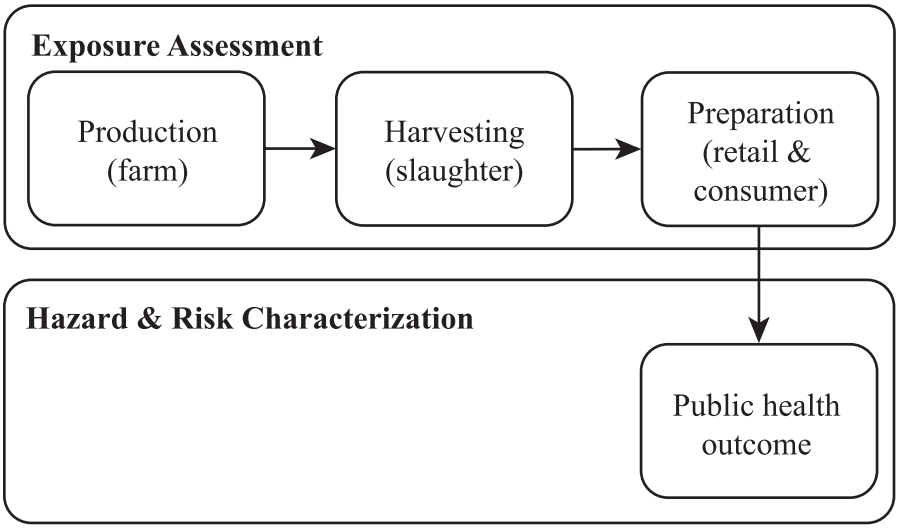

Figure 3. General Structure of the USDA/FSIS Risk Assessment for *E. coli* O157:H7 in Ground Beef (USDA/FSIS, 1998b)

restricted focus of the exposure assessment on contamination level (that thus fails to characterize exposure patterns specific to population subgroups), risk characterization eventually has a limited ability to consider specific health effects in particular individuals. This shortcoming is common to risk assessments for both foodborne and waterborne pathogens.

Conclusion

Public health decisions need to be made in the face of provisional, and sometimes contradictory, scientific knowledge. In this context, risk assessment attempts to address a real need of policy makers by systematically collecting, analyzing, and inferring from available information (however imperfect that might be). It builds upon classical scientific disciplines, augmenting rather than working at cross-purposes with these disciplines. However, while scientists often focus on positive evidence (what we know), the characterization of uncertainty — i.e., how uncertain we are concerning what we know — should be of as much importance to a risk manager. A risk assessment will thus discuss at length its limitations and assumptions in a desire to document the process fully. This is in conformity with the complexity of the question posed and the limited data rather than an inherent flaw of the approach. Finally, it is noted that risk assessment can also be an important predecessor for conducting cost-benefit analyses or developing Hazard Analysis and Critical Control Points (HACCP) plans, all of which provide further information for developing risk management alternatives.

Risk assessment methods have increasingly been applied to microbial food safety hazards in the past several years. In particular, several large projects have been conducted in the United States. It should be recognized that the approaches and methods used in those studies draw on the experiences from chemical risk assessment and on previous research related to waterborne pathogens. While the application of risk assessment to foodborne microbial hazards offers a considerable body of experience in the practice of microbial risk assessment, methodological innovations are still needed. In particular, the characterization of the occurrence of debilitating health effects, which tend to differentially affect specific population subgroups, is an increasingly recognized need. The ability of the current approaches to address such a need adequately is limited, but viable alternatives are still missing.

For instance, in hazard characterization of foodborne pathogens, risk assessors are confronted with a complex interaction of host, microorganism, and food vehicle factors. Nonetheless, knowledge of the single factors as well as the understanding of their interaction, is limited. Confronted with this uncertainty, risk assessors have often opted for pre-made, one-fit-all solutions. Whereas there is no hard evidence indicating that such an approach is unsuitable, no positive proof of its soundness exists either. If one were to characterize specific health effects in particular population subgroups (which is a recognized need), a dose-response relationship in susceptible subjects for low-dose exposure of highly pathogenic strains would be necessary. Under experimental conditions, this would imply conducting an extensive set of human feeding trials with large numbers of susceptible subjects — hardly a feasible option due to ethical and cost concerns. This creates the need for novel approaches that utilize information obtained from observational studies, in particular epidemiological studies. Table 2 summarizes the potential relevance of epidemiological studies as information sources for microbial hazard characterization.

Table 2. Potential Contribution of Epidemiological Studies as Information Source for Microbial Hazard Characterization

Types of epidemiological studies	Components of hazard characterization			
	Dose	**Pathogen**	**Host**	**Food**
Descriptive studies				
-- Case series (e.g. outbreak investigation)	+	++	-	++
-- Cross-sectional (e.g. surveillance)	-	++	+	+
Analytical studies				
-- Case-control & cohort	-	+	++	+
-- Clinical trial	++	++	-	-

Key: - = not useful, + = useful, ++ = very useful

Finally, an emerging food safety issue potentially linked to irrigation and water contamination involves fresh produce. Examples of outbreaks are: lettuce and *E. coli* O157:H7 (Ackers et al., 1998), apple cider and *Cryptosporidium parvum* (CDC, 1997), and lettuce/tomatoes and *Giardia lamblia* (CDC, 1989). Such outbreaks highlight the increasingly blurred distinction between waterborne and foodborne pathogens. In terms of public health, the two transmission modes merely reflect two different pathways along which a fecal-oral contamination can occur. For several pathogens, both scenarios as well as others (e.g., person-to-person contact) should ideally be considered. Microbial risk assessments for waterborne and foodborne pathogens seem destined to grow increasingly close methodologically. However, fully coordinated applications for water and food safety may be limited at the federal level due to differing agency mandates. Were such institutional hurdles to be surmounted, the assessment of aggregate risks — i.e., the risk caused by a pathogen over different exposure scenarios — might become reality.

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SECTION VI

Microbial Risk Management

Pathogen Reduction Requirements of 40 CFR Part 503: Their Origin, Evaluation, New Reduction Approaches

Pathogens In Biosolids: Risks and Regulations

Regulations and Strategies for Controlling Pathogens in Biosolids in the United Kingdom

Pathogen Reduction Requirements of 40 CFR Part 503: Their Origin, Evaluation, New Reduction Approaches

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Background

The Part 503 regulation (U.S. EPA 1993) was preceded by an earlier regulation (U.S. EPA 1979) that controlled the use of wastewater sludge on the land. The Part 503 rule built on parts of the earlier rule, so it is necessary to show this connection for an adequate understanding of the use of certain terms and requirements in the Part 503 rule.

Before establishing the 1979 rule, the Office of Solid Waste of the U.S. EPA (EPA) conducted an investigation of the state of the art of sludge treatment and disposal. It concluded that there was essentially no documented evidence of disease incidence when sewage sludge was applied to the land, provided processes such as adequate anaerobic digestion of the sludge followed by application at reasonable rates to land were practiced. These practices did not eliminate pathogens from the sludge but the net result appeared not to create a health risk. EPA then constructed the regulation so as to codify good practice by standardizing processing conditions for stabilization processes that had to be adhered to or exceeded, and set up rules concerning crops to be grown, grazing restrictions and access limitations that added additional safeguards that minimized health risk. The processes identified, called "processes to significantly reduce pathogens" (PSRPs), were required to stabilize the sludge so as to minimize attractiveness to vectors as well as reduce pathogens. For those facilities that desired freedom from restrictive requirements concerning access and crops grown, a second classification, called "processes to further reduce pathogens" (PFRPs), was established. These processes still had to reduce attractiveness to vectors, but they also had to produce a sludge with no detectable viable *Salmonella*, viruses or helminths. A third class of processes, called "add-on processes", was also established. These processes only reduced pathogens. They could not be used alone but had to be used in conjunction with a PSRP. The combination of an add-on process and a PSRP had the same effect as a PFRP.

Only a limited number of methods of treatment to reduce pathogens and vector attraction were named in the 1979 regulation. This proved to be very restrictive. To provide room for new developments, the 1979 regulation allowed for processes demonstrated to be equivalent to PSRPs and PFRPs. Subsequently, EPA instituted its Pathogen Equivalency Committee (PEC) to evaluate processes brought to its attention and recommend to regulatory staff whether the processes were "equivalent" to the named processes. The PEC membership consists of EPA engineers and microbiologists with training and experience in the health effects of pathogens and their removal or inactivation by treatment processes.

When the Part 503 regulation was written, the pathogen reduction processes were separated from the vector attraction reduction (VAR) processes. Specific pathogen reduction goals (described below under the heading *Class A and B Requirements in the Part 503 Regulation*) were set, so there was no need to specify particular pathogen reduction processes. In principle, this provided a much greater opportunity to come up with ways to meet the challenge of pathogen reduction. Unfortunately, standards could not be established easily for VAR, so either named processes were utilized for this requirement or specific testing procedures were required to establish adequate VAR. There is no provision in the Part 503 regulation for "equivalent" VAR processes.

Despite the fact that pathogen reduction standards are specified in the Part 503 regulation, PSRPs and PFRPs are also included. The rationale for including them is that municipalities all over the United States were using them and their utility had not been disproved. To include them in the new regulation, they were stripped as far as possible of the features that caused them to be VAR processes while retaining their pathogen-reducing ability. Thus, for example, to meet the Class A or B pathogen-reducing requirement, one could either meet the specified microbial reduction, use one of the stripped PSRPs or PFRPs, or use a process equivalent to them.

It is important to be aware of what pathogens were of concern to the writers of the 1979 and 1993 regulations. The concern was for disease-causing organisms for which wastewater and sludge constitute an important route for dissemination (World Health Organization 1981; Kowal 1985). Some microorganisms found in sludge were considered of minor concern. For example, spores of *Clostridium perfringens* are abundant in sludge, but their presence in sludge is considered to cause an insignificant increase in the risk of disease if the sludge is used on land, which already contains great numbers of these spores. It should also be noted that the regulation is concerned about enteric viruses and enteric bacteria. Enterovirus densities and *Salmonella* species or fecal coliform densities are used as surrogates for these organisms.

Emerging Pathogens

In the years since the first regulation was issued in 1979, there has been concern about pathogens other than those specifically measured or accounted for by the density measurements of salmonellae, enteroviruses and helminth eggs. One of the obvious omissions was lack of consideration of the hepatitis A virus. Infectious hepatitis is transmitted through fecal discharges. At the time of the 1993 regulation, there was no economical way to quantify its infective potential in sludge. It was hoped that the enterovirus determination would be an adequate indicator for the potential presence of this virus. Funding was so limited that investigations of the effect of processing on this virus were never started. There is some justification for this lack of activity. Virtually all cases of infectious hepatitis are traceable to inadequate hand-washing or poor sanitation in the home. Infectious hepatitis pockets are associated with poverty and lack of education about good sanitation and are not associated with use of sludge on land.

The discovery of the serious nature of infections with the protozoa *Giardia* and *Cryptosporidium* spp. has created concern about infection with these organisms by ingestion or inhalation of sludge products. The route of transmission of these protozoa also is through fecal discharges. Still, the cases that occur seem to be primarily associated with drinking water or recreational water contaminated by animal or human fecal wastes. Until recently, the work of Stadterman et al. (1995) gave some assurance that biological treatment greatly reduced densities of some protozoa. These authors reported that *Cryptosporidium parvum* oocysts were inactivated relatively quickly by anaerobic digestion at 37°C. Their data show first order reduction with time. Reduction in four hours was 90%. A rate constant is easily calculated from these data, giving a value of 13.8 da^{-1}. The fractional reduction in organism density that would occur across a continuously fed continuously mixed digester can be calculated using this rate constant. For a digester with a hydraulic residence time of 15 days, the ratio of output density to input density would be 0.005. This is about the same as occurs with fecal coliform. Recent studies by Australian authors (Gibbs et al. 1998) have raised the possibility of survival of protozoan cysts even under as severe an exposure as composting. Gibbs et al. conducted a risk assessment that shows substantial risk of infection. However, in all of their studies, these investigators did not determine viability or infectivity of the cysts. Obviously, follow-up work is in order, but reporting results without knowing the biological significance does not seem valuable.

There is concern about blood-borne pathogens such as the human immunodeficiency virus (HIV) and hepatitis B and C. Lue-Hing et al. (1999) indicate that potential for infection by HIV from sewage and sludge is remote, particularly compared to other routes. Potential for HIV infection probably has the lowest risk.

The 1979 and 1993 regulations focus on salmonellae as the pathogenic bacteria of greatest concern. Use of salmonellae as a surrogate for the rate of decline of pathogenic bacteria generally makes some sense, but salmonellae are definitely not a good indicator of the presence of other pathogenic bacterial species. More work clearly is in order to determine density levels and response to treatment processes of other bacterial pathogens such as *Shigella* spp. and *E. coli* 0157-H7. This concern is especially important, because of our requirement that a Class A sludge meet either a fecal coliform density of 1000 MPN/g *or* a *Salmonella* spp. density of less than 3 MPN/4g. Compost facilities frequently have trouble meeting the fecal coliform requirement so they turn to the *Salmonella* spp. determination. Essentially they are using salmonellae as an indicator, and this is a dubious procedure.

Class A and B Requirements in the Part 503 Regulation

Class A: The requirements for sludge to be classified Class A with respect to pathogens are spelled out in six alternatives. Common to every alternative is the requirement that the density of *Salmonella* spp. be reduced to less than 3 MPN/4g of solids, or the fecal coliform be less than 1000 MPN/g of solids. The six alternatives, briefly stated, are:

- Alternative 1. Meet the time-temperature requirements stated in the regulation. These requirements were established from FDA requirements for eggnog, data from German sources, and other data collected during composting experiments in the USA (U.S.EPA 1992c). The time/temperature conditions are specified for finely divided particles of sludge suspended in fluid such as hot gases or oil, aqueous sludges greater than seven percent solids, and aqueous sludges less than seven percent solids. For the latter case, the time of treatment in days must be greater than 10,070,000 divided by 10 raised to the power 0.1400 times temperature in degrees Celsius. The time period cannot be less than 30 minutes and the temperature cannot be less than 50°C.

- Alternative 2. This alternative is based on an equivalency ruling given before 1993 to a proprietary process (N-VIRO). The pH is raised to above 12 for greater than 72 hours, the temperature to above 52°C, and, after the 72 hours, the treated sludge is air-dried to 50 percent solids or greater.

- Alternative 3. The sludge is first analyzed for viable helminth eggs and enteric viruses. If these organisms are not present (less than one ovum and less than one plaque-forming unit (PFU)/4g of solids), the process is Class A only until the next monitoring period. If the organisms are present in the feed but not in the product, the product produced in the future is Class A for pathogens provided that the process operating conditions are consistent with those utilized during the test.

- Alternative 4. The sludge is analyzed for viable helminth eggs and enteric viruses. If they are below one viable ovum and one virus PFU/4g of solids, the material is Class A with respect to pathogens.

- Alternative 5. The sludge is treated by a PFRP as described in Appendix B of the regulation. The PFRPs are: composting, heat drying, heat treatment, thermophilic aerobic digestion, high-energy electron beam irradiation, and gamma ray irradiation (operating conditions for these processes are specified in regulation Appendix B). The add-on processes of the 1979 regulation are included as PFRPs in the Part 503 regulation.

- Alternative 6. The sludge is treated by a process equivalent to a PFRP.

Class B: The requirements for a sludge to be classified Class B with respect to pathogens are spelled out in three alternatives:

- Alternative 1. The geometric mean fecal coliform density (either MPN or colony forming units (CFU)/g) of seven samples shall be less than 2,000,000.

- Alternative 2. The sludge is treated by a PSRP as described in Appendix B of the regulation. The processes are: aerobic digestion, air drying, anaerobic digestion, composting (less stringent thermal requirements than the PFRP), and lime stabilization (operating conditions for these processes are specified in regulation Appendix B).

- Alternative 3. Sludge is treated by a process equivalent to a PSRP.

There are eight site restrictions that must be followed for application of Class B sludge. These are:

1. Above-ground food crops that touch the soil are not harvested for 14 months after sludge application.

2. Below-ground food crops are not harvested for 20 months after application if sludge has remained on the soil surface for four months or more prior to incorporation.

3. Below-ground food crops shall not be harvested for 38 months after sludge application if the sludge has been on the surface of the soil for less than four months before incorporation.

4. No crops shall be harvested for 30 days after application of sludge.

5. Animals shall not be allowed to graze for 30 days after application of sludge.

6. Turf grown on land where sludge is applied shall not be harvested for one year after application when it is to be placed on a lawn or other site with high potential for public exposure unless otherwise specified by the permitting authority.

7. Public access is restricted for one year if there is high potential for public exposure.

8. Public access is restricted for 30 days if there is low potential for public exposure.

Effectiveness of Class A Requirements

The effectiveness of the Class A alternatives has to be considered individually since they are all different:

- Alternative 1. The data supporting this alternative have been provided in the technical support document for the regulation (U.S.EPA 1992c). The alternative is based on data from German publications, German guidance, FDA requirements for pasteurization of eggnog, and U.S. data on pathogen reduction in composting. Practitioners using this alternative do not have to conduct analyses for viable helminth eggs or enteric viruses. However, they must satisfy a fecal coliform or *Salmonella* spp. density requirement. The thermal equations are believed to be conservative, but no trials have been run after the regulation was published to test this belief. Probably the most commonly used process that depends on Alternative 1 is aerobic thermophilic digestion. There have been no reports of difficulty in achieving the desired fecal coliform or *Salmonella* spp. densities with this process. Alternative 1 is being used in combination with both aerobic and anaerobic thermophilic digestion. All of these schemes use batch treatment of the sludge. No processes using continuous plug flow or a series of complete-mix vessels, using this alternative, have been approved by EPA's PEC.

- Alternative 2. Like Alternative 1, only fecal coliform or *Salmonella* spp. have to be quantified and be below specified limits. However, N-VIRO Corporation (www.nviro.com) has required that for large plants, enterovirus and viable helminth egg densities be determined. The records of an independent laboratory show that they have never seen densities in the product exceeding the density requirements of the regulation (private communication with Dr. Faulmann of Biocheck Laboratories, Toledo, Ohio, 2001).

- Alternative 3. This alternative is troubling. It would be possible for a process with only a minimal ability to reduce virus and viable helminth egg densities to qualify as a Class A process if low densities of these organisms were present in the feed at the time of the monitoring test. An astute regulator could insist on assurance that the density levels at the time of testing be at least on the high side of what the plant typically encounters during the year, but there is no assurance that he/she would be astute or even that his/her desires would prevail. It is fortunate that the fecal coliform density must be below 1000 MPN/g or the *Salmonella* spp. density below 3 MPN/4g. At least the process must reduce bacterial pathogen levels. Fortunately, developers of a new Class A process are usually not interested in utilizing Alternative 3, because it is site-specific. They prefer Alternative 6 (PFRP equivalency) since this designation is normally not site-specific. Pursuing Alternative 3 involves an economic risk because the facility must be tested on a full scale. If the process should fail, the investment in the process would be wasted. Secondly, the cost for microbial analyses would likely be very high. To certify the process, it must be shown that viruses and viable helminth eggs were present in the feed and not in the product. Proving this could require analysis of many samples. Most wastewater processes have long residence times, making it difficult to relate input to output. Also, densities of both helminth eggs and viruses in the feed are usually highly variable, ranging from absence to densities of a 1,000/4g of solids. If the organisms are sporadically absent, it is especially difficult to show conclusively that they have been destroyed.

- Alternative 4. This alternative is sometimes called the "orphan" sludge alternative. It applies generally to a mass of sludge which has been subjected to conditions that are very likely to produce Class A pathogen reduction (e.g., long storage, or composting where conditions were not properly documented). The mass is sampled properly and virus, viable helminth eggs, and *Salmonella* spp. or fecal coliform densities are determined. If densities are below the values specified in the regulation, the material is Class A with respect to pathogens. Obviously, all such material has met

the requirements of the regulation. This alternative has been used frequently, sometimes for huge batches of sludge or sludge product. Adequacy of sampling of huge volumes could be an important issue.

- Alternative 5. Any of the seven PFRPs listed in Appendix B of the regulation may be used at the conditions specified. Of these processes, composting — both windrow and static pile — is extensively used, as well as heat drying. It is not necessary to measure viral and viable helminth egg densities, so data on reduction of these organisms from operating facilities are generally not available. Apparently, the *Salmonella* spp. requirement is easily met by composting facilities, although the regulatory limit for fecal coliform densities is often exceeded. Heat drying usually easily meets either requirement. There are numerous heat treatment facilities (liquid sludge is raised to high temperatures) that would easily meet all pathogen reduction requirements. However, they generally do not meet the requirement for Class A pathogen reduction, because the pathogen-reducing process is the last processing step. With this processing sequence, the product is left with no living vegetative bacterial cells that would normally provide competitive inhibition if contamination with bacterial pathogens occurred. Clements (1983) reported the Swiss experience where pasteurization after digestion was practiced. Most of the products were badly contaminated with pathogenic bacteria. When facilities switched to pasteurization before digestion, the problem disappeared. For this reason, the regulation requires that pathogen reduction either precede or be concurrent with the VAR step.

- Alternative 6. Any process equivalent to a PFRP only needs to meet the requirement to reduce *Salmonella* spp. or fecal coliform densities to below the specified values, and to operate the process at the conditions used in the demonstration. The revised "White House" document — so-called because of the photograph of the White House conspicuously displayed on the cover (U.S.EPA 1999) — lists nine equivalent PFRP processes (sometimes called E-PFRPs). These processes (and the names of the company developing the process) are: Two-stage sludge stabilization (CBI Walker); Autothermal thermophilic aerobic digestion (Fuchs Gas & Wassertechnik); Within-vessel composting (International Process Systems); Indirect drying (K-F Environmental Technologies); Two-phase thermo-meso anaerobic digestion (Lyonnaise des Eaux); Alkaline stabilization (ATW Inc.); Advanced alkaline stabilization (N-VIRO Energy Systems); OxyOzonation (Synox Corporation); and Microbiological conditioning and drying (Ultraclear). Most of these processes met the requirements of Alternative 1, the time-temperature relationship. The companies involved petitioned the PEC for a declaration that their processes were "equivalent" to a PFRP. For three of the processes, developers had to satisfy the PEC that adequate microbial reductions were achieved. These processes have been proved effective to the satisfaction of the PEC.

Effectiveness of Class A alternatives: If the alternatives meet the stated requirements of the regulation, there is virtually no risk from the pathogens of concern (not considering "emerging pathogens"). Processes do not always operate as planned, and product is not always diverted to a less stringent option, such as a Class B application when off-specification product is suspected.

Effectiveness of Class B Requirements

Class B requirements include the combined effect of partial reduction of pathogen densities by processing and restrictions as to types of crops, access limitations, and limitations between time of application and removal and distribution of crops. Class B pathogen reducing processes have been found to reduce viral and bacterial pathogen densities by factors between 10 and 100 (U.S.EPA 1992c). Reports of viral densities (U.S.EPA 1992a) vary widely as do bacterial densities (U.S.EPA 1991b). However, upper values in domestic sludges can be as high as 1000 PFU/g of solids for enteroviruses and as high as 1000 MPN/g for *Salmonella* spp. Under some circumstances then, it is likely that these pathogens will be present in the treated sludge. The combination of processing and restrictions must reduce risk to negligible levels. The two effects are considered separately below.

Effectiveness of the processes: The effectiveness of the Class B alternatives is as follows:

- Alternative 1. The pathogen reduction requirements are to reduce the fecal coliform content to 2 million MPN or CFU/g of dry solids in the sludge. The fecal coliform density of the solids in domestic wastewater is approximately 200 million MPN or CFU/g of solids. The standard was set with an appreciation of the pathogen-reducing performance of typical well-operated solids treatment processes. In the previous regulation (U.S.EPA 1979), the standard was set on the basis of

typical reductions across the sludge treatment process. That type of standard could be deficient if, as sometimes occurs, the pathogen burden is high or low. For example, a primary sludge tends to have a high density of pathogens (based on indicator organism count), whereas an extended aeration sludge is much lower. The risk from contact with the solids in wastewater is reduced (on a solids basis) by a factor of 100. If this requirement is met, the process has met its expectations.

- Alternative 2. The microbiological reductions of the five PSRPs of the 1979 regulation were established by collecting data on well-operated processes that reduced pathogens and produced a product that did not continue to putrefy after application. Virus, salmonellae, and fecal indicator organism declines were observed. Conditions that produced reductions of about two $\log_{10}$ for fecal coliform, about one $\log_{10}$ for viruses and about 1.5 $\log_{10}$ for salmonellae were selected for each of the processes. In some cases, pathogen reductions were greater than this (e.g., with lime stabilization, the amount of lime required to prevent future putrefaction produced a much greater reduction in fecal indicator organisms and salmonellae, and probably viruses). Any of the five PSRPs listed in Appendix B of the regulation may be used at the conditions specified. No microbiological measurement need be carried out if the processing conditions are met. The requirements of Alternatives 1 and 2 are consistent. There is ample information in the literature that anaerobic digestion that meets or exceeds the PSRP requirements will meet the 2,000,000 fecal coliform density requirement (Stukenberg et al., 1994; Shimp et al., 1994; Ponugoti et al., 1997; Lucero-Raminez and Malina, 2000). Aerobic digestion generally meets the fecal coliform requirement if carried out at specified conditions (Shimp et al. 1994; Ponugoti et al. 1997). Lime stabilization produces a much greater reduction in fecal coliform content than the digestion processes (U.S.EPA 1992c). The air-drying PSRP is generally used at facilities that treat wastewater by the extended aeration process. The sludges removed from extended aeration plants very nearly meet the 2,000,000 fecal coliform/g requirement (Farrell et al. 1990; Ponugoti et al. 1997) so there is little doubt that the additional air-drying will reduce the measurement below the required level. Composting to produce a Class B product is rarely used. The elevated temperature that occurs in this process is expected to reduce the fecal coliform densities to levels much below the Part 503 requirement. Composting processes generally must meet a consumer quality requirement, so a high standard of performance is expected.

- Alternative 3. Several site-specific processes equivalent to a PSRP (E-PSRP) were established during the period that the 1979 regulation applied. A partial listing is found in the original "White House" document (U.S.EPA 1992b, Table 5-2). Most of these were site-specific processes. The change in the Part 503 regulation that stripped PSRPs and PFRPs of the requirement for vector attraction affects these processes. Doubtlessly, local regulators have resolved any issues created by this change. The revised "White House" document (U.S.EPA 1999) describes a requirement (p. 97 in U.S.EPA, 1999) for an "equivalent" PSRP (E-PSRP) that sets a more stringent standard than is required by Alternative 1 of the regulation. A facility could quietly use Alternative 1, but if it wants an officially recognized E-PSRP, it will likely have to satisfy slightly more stringent fecal coliform and fecal streptococcus reductions, and show a correlation between pathogen reduction and indicator organism reduction if the process is not a typical digestion process. The stricter requirements seem appropriate, but the inconsistency should be recognized. Table 11.2 in the revised "White House" document (U.S.EPA 1999) lists two E-PSRP processes that presumably have satisfied this stricter requirement.

Effectiveness of the Class B restrictions: As noted above, sludges reduced in pathogens by Class B processes are reduced in enteric virus and pathogenic bacteria densities by between one and two $\log_{10}$. Viable helminth egg densities are reduced very little, even by the lime stabilization PSRP. The restrictions are expected to reduce the density of pathogens leaving the site on products, fomites, and vectors so that they pose a minimal risk of causing disease. Evidence that the restrictions achieve this goal is limited. The most determined effort to demonstrate the effectiveness of the restrictions was an epidemiological study conducted by Dorn et al. (1985) at Ohio State University. Illness rates in families living on farms on which Class B sludge was applied were compared with rates at farms where there was no sludge application. Results showed no significant difference in illness rates. Although this result is encouraging, its conclusions are open to question. It would be expected that a family whose state of health was being carefully followed because Class B biosolds were being applied to their farm would be more careful about handwashing and other aspects of personal cleanliness. Thus, they might be healthier than a farm family subjected to the same health scrutiny but without biosolids application to their land. A blind study in which half the farms

received Class B biosolids and the other half received Class A biosolids would avoid this obvious defect. Unfortunately, such a study would be extremely expensive.

The restrictions generally place a time barrier between application and possible exposure. There is ample evidence that densities generally are greatly reduced if the restrictive requirements are met (Sorber and Moore, 1987), but there are exceptions. Uncontrolled or unknown variables, such as degree of stabilization of the sludge, sludge loading to the soil, rainfall, shading, average temperature, and soil composition may influence rate of die-off. Viruses fortunately decline substantially and irreversibly. Helminth eggs die off on the surface of the soil but survive for long periods if under the soil or in moist shaded locations. Pathogenic bacteria decline as well, but there is potential for regrowth, depending on the aforementioned variables. Sorber and Moore (1987) claim that the available data show that only members of the coliform group regrow. However, Yanko et al. (1978) report that salmonellae occasionally re-grew on plots to which sludge had been applied. The long interdiction periods for access and for above-ground crops are probably protective. Pathogenic bacteria probably survive in some cases in substantial numbers for more than a month (the period of interdiction for grazing), but possibly not at sufficient densities to constitute an infective dose.

Intuitively, it seems that the greatest risk from land application of Class B sludge is related to unusual or unexpected events. An example would be a sudden rain event that occurs soon after sludge is applied to a field. Another high-risk circumstance is disking or plowing on a windy day too soon after sludge application. A frequently overlooked circumstance is the transport of sludge from a field into a farm home on the coats of household cats and dogs. Many dog owners have been dismayed by the (to us) odd behavior of dogs that seem to enjoy rolling in excreta. Farm owners should be aware of the possibility of this kind of behavior and at least keep their pets that run free from entering the home.

The EPA has published a series of risk assessments, which consider use and application of both Class A and Class B sludges (U.S.EPA 1989, 1991a, 1991b, 1992a). These publications show low risk of disease from these practices. These risk assessments, of course, depend on the quality of the data, which is inadequate in most cases. They also cannot easily quantify the effect of unusual or unexpected events.

Cheng et al. (1994) report on an especially relevant investigation conducted for a period of three months at a land farm in Louisiana. At this site, aerobically digested waste activated sludge is pumped to a lagoon and periodically sprayed on the land surface. A hay crop is grown and sold locally. Drainage enters the site primarily at one point. Collected drainage is discharged into a nearby stream. The authors measured fecal coliform and fecal streptococcus densities in the lagooned sludge, in the incoming water draining onto the property, in the drainage leaving the site, and in the receiving stream up- and downstream from the point of entry of the drainage water from the site. Summary data for these sampling points are shown in Table 1.

The data show that the drainage is enriched in fecal coliform as expected. Surprisingly, the density downstream in the receiving stream averages even higher than the density at the drain outlet, possibly because of the enrichment of the stream bed by settling of solids and nutrients from the drainage stream or possibly by additional drainage points. The data show a correlation between rainfall incidents and increase in density at the drainage outlet, although in some cases spikes in fecal coliform occurred unexpectedly and did not occur after some heavy rainfalls. It should also be pointed out that the sludge being sprayed had a fecal coliform density of only 98,459 CFU/100 mL. The reduction between sludge in the lagoon and the drainage was by a factor of 10. A typical sludge of about 4 percent solids that just meets the fecal coliform

Table 1: Average Fecal Coliform Densities at Sampling Points

Sampling point	Ave. FC density (CFU/100 ml)	Ratio (x/inlet)
Drainage inlet	1509	1.0
Drainage outlet	9860	6.5
Upstream	2831	1.9
Downstream	14690	9.7
Storage lagoon	98459	65.2

requirement of the regulation would have 8,000,000 CFU/100 mL. If the factor of reduction were the same, the drainage would have a fecal coliform density of 800,000 CFU/100mL, an alarmingly high figure.

An important question is whether lowering the regulatory requirement to something below 2 million MPN or CFU/g of solids would make a significant reduction of the risk of disease from application of Class B sludge. There is not much doubt that reducing the required fecal coliform density by a factor of ten would reduce risk from bacteria and viruses, but not from helminth eggs. However, if the risk is already very low, this is a costly step with minimal gain. It would certainly lower the risk from unexpected events, such as washout of freshly applied sludge by a heavy rainstorm, but there may be less costly approaches than requiring the entire industry to meet a much more difficult standard. A lower cost approach would be to increase the requirements for preventing runoff. A start might be to increase the buffer zone, which only requires that sludge not be applied within 10 meters from "waters of the United States" (40 CFR 122.2). An increase in the width of the buffer zone and a requirement that the buffer zone be vegetated would be very helpful. A requirement to plow in or inject sludge would greatly reduce risk from runoff, but would increase danger if root crops were grown.

Another factor to consider is the requirement to reduce vector attraction. If, for example, only pathogens were reduced, the sludge would still be a high-energy food and a good substrate for microbial regrowth. There is ample evidence that when bacterial densities are reduced and vector attraction is not sufficiently reduced, regrowth of bacterial pathogens can occur, sometimes to very high densities. The effect of the specified VAR requirements on risk of bacterial regrowth and actual attraction of vectors is poorly understood. Upgrading our understanding of VAR and possibly changing some of the requirements would doubtlessly improve the regulation.

Monitoring Requirements

The monitoring frequency of the regulation for pathogens or pathogen surrogates range from once a month for large plants (greater than 15,000 tons dry weight of sludge solids per year) to once a year for small plants (less than 290 tons dry weight per year). During the intervals between tests, the processes should be run under the same conditions as were maintained during the testing period. Pathogen reductions are expected to remain the same. To anyone familiar with industrial quality control, these frequencies are incredibly low, so low that it is difficult to believe that they are protective. The idea behind the rule is that if process conditions remain the same, the reduction in pathogens will remain the same. The deficiency in this approach is that for some processes, our knowledge of the pathogen reduction response to changes in process variables or to variables that are unknown or not under control is generally inadequate. An example of a process where the approach is adequate is the effect of gamma radiation. If the dose is one megarad (10 kGray), all microbial life will be destroyed. There is one easily monitored process control! Electron beam irradiation is less of a sure thing, because dose depends on the thickness of the sludge layer being irradiated. If the process is windrow composting, the initial moisture, the number of turnings, season of the year, sunlight intensity, composition of the mixture (e.g., its ammonia content), site and equipment cleanliness, and the atmospheric conditions could all have an influence. The only way to assure that pathogen reduction is satisfactory is to check pathogen reduction at some reasonable frequency, which is more frequently than once a month.

It should be mentioned that the United States is not alone in recommending inadequate times for checking that a process is performing properly. Brinton (2001) reports that recommended checking for product quality for composting facilities is twelve times a year in Germany for facilities producing over 12,000 tons per year. The Netherlands recommends one time for each 5,000 tons or a minimum of five times a year. Other countries make similar or even less stringent recommendations. The desire of the regulators not to impose expensive monitoring requirements is understandable but risky. This situation can be partially addressed by developing lower cost monitoring methods (thus reducing the cost of more frequent monitoring), and educating regulators to the status of knowledge of various processes so that they can exercise closer scrutiny to those processes that need it. One improvement would be to schedule the monitoring for a period of the year that is most stressful for the process (e.g., examine composting facilities in the winter); another would be to make monitoring visits on short notice.

The quality of process control and of records kept during the periods between monitoring visits should be high. Monitoring of operations by plant personnel should be frequent enough to detect and correct off-specification operation before harm is done. Material produced during off-specification operation should be diverted to a less stringent application (e.g., to landfill or Class B use) or reprocessed. Falsification of records, of course, is a criminal act and probably occurs rarely. However, there should be measures taken against a facility if the regulator observes that process control and recordkeeping are poor.

A glaring deficiency of the 1979 and the 1993 regulations is the paucity of descriptions of the processes and methods to be used. It is difficult to monitor a process if its description is inadequate. This situation is in marked contrast to the air pollution regulation where test methods are described in complete detail. This failure to provide enough detail has resulted in inadequate descriptions of almost every process, even the simpler ones.

Developing Class A Processes

This section will emphasize new methods or variants of existing Class A processes. Processes in common use are mentioned here but are not discussed below. Composting is probably the most used of the Class A processes. The deep-pile method and the windrow method are both used. They are existing PFRPs and they meet EPA's thermal equation as well. Heat drying is another process that is commonly practiced. Direct and indirect heating processes are used; some variants are discussed below. There are numerous facilities that use unslaked lime or lime-like addition with added or self-generated heat to produce a Class A product. A number of two-stage aerobic thermophilic digestion facilities have been installed in the United States and Canada (Deeny et al. 1991). There are a limited number of two stage thermophilic-mesophilic anaerobic digestion processes either installed or being constructed (U.S.EPA 1999; Currie, 1997). These processes will not be discussed explicitly in the following text.

Time-temperature These processes satisfy the thermal relationship of EPA's Class A Alternative 1. The relationship applies to processes that assure that every portion of the sludge receives the desired thermal treatment. It applies to batch and plug-flow processes. Examples are discussed below.

- Heat drying. A novel approach to heat drying is to filter the sludge in a diaphragm filter press, which produces a low moisture cake (ca. 40 percent solids). Steam is passed through the core of the plate (not in contact with the sludge cake) and a vacuum is drawn on the cake. The cake can be dried to 90-plus % solids. The process (DRY-VAC Environmental, Inc., Rio Vista, CA) can be operated to be certain that the cake meets the thermal equation of Alternative 1. Because the cake is dry enough, it meets Vector Attraction Requirements 7 or 8 of the 1993 regulation. Since this process meets Alternative 1, it may not be necessary to get Pathogen Equivalency Committee (PEC) approval, although this would be a wise step to take.

- Thermophilic aerobic-anaerobic digestion. The CBI Walker process has been mentioned above and involves dual digestion (aerobic first stage using oxygen, mesophilic anaerobic digestion in second stage – Lotepro Corp., Valhalla, NY). Developers had not approached the PEC as of the date of this workshop. This process has potential to be Class A with increased operating temperature in the first stage and design changes to prevent bypassing. The aerobic stage may present design problems for large plants.

- Thermophilic aerobic digestion. A single stage process with a ten-day residence time is listed as a PFRP. There are no users to this writer's knowledge. This is fortunate because the process description in the regulation is inadequate. The description would allow complete-mix operation, which would not satisfy the thermal equation. It is likely that if operated as a complete-mix system, the process would not achieve a fecal coliform density of less than 1000 MPN/gram although it might meet the alternative of less than three MPN *Salmonella* spp./4g.

- Thermophilic-mesophilic anaerobic digestion combinations with intermittent feeding. The Lyonaisse process (Huyard et al. 2000), approved as an E-PFRP, uses an anaerobic thermophilic acid-phase digester in series with a mesophilic digester. The process is approved but must be demonstrated on a full scale to obtain a non-site-specific designation. PEC approval was needed because the period of batch treatment was for a much shorter time period than allowed by Alternative 1. The Orange Water and Sewer Authority (OWASA, Raleigh, NC) has installed a multiple stage anaerobic digestion train in which one thermophilic stage achieves the conditions of EPA's thermal equation by intermittent feeding. An earlier paper (Farrell et al. 1996) suggests that the Class A requirement could be met by partial pathogen reductions in a series of digesters. However, the OWASA installation carries out the entire time-temperature reduction period in a single digester.

- Continuously fed thermophilic anaerobic digestion. At Annacis Island in Vancouver, British Columbia, there are several continuously fed and continuously mixed thermophilic digesters connected in series. Exceptionally high reductions in fecal coliform densities are achieved. If pathogen

decline with temperature is assumed to be first order, calculations based on complete mixing, using a reaction rate constant calculated from the EPA equation, show that such systems can produce results equivalent to batch thermal treatment. This approach has not been approved by EPA's PEC. They require demonstration on at least a pilot scale that the pathogens of concern are destroyed, probably followed by tracer tests on a full scale to show equivalent or longer residence times than in the pilot-scale unit.

- Continuously fed mesophilic-thermophilic anaerobic digestion. There are a number of installations that use a thermophilic first stage anaerobic digester (either an acid-phase or a short residence time thermophilic stage) followed by a mesophilic second stage. Claims have been made for Class A performance. The PEC has not been approached for approval. A demonstration of performance would be needed. It is unlikely that these processes will achieve Class A performance unless some special designs are developed that avoid bypassing.

- Pre-pasteurization. Pre-pasteurization before mesophilic anaerobic digestion was practiced in Europe as a batch process many years ago after the recognition that post-pasteurization was a very risky process because of the frequent catastrophic regrowth of *Salmonella* spp. The process has not attracted interest in the United States, although it is now being offered as a batch process by an equipment manufacturer. Use of a continuously fed plug-flow pasteurization vessel is possible, but there are no installations. At a minimum, the flow distribution would have to be verified to assure that bypassing did not occur.

- Post-pasteurization. Researchers at the University of Washington (Ward et al. 1999) have demonstrated that it is possible to post-pasteurize sludge and not have a problem with regrowth of bacterial pathogens. It would be possible to combine such a process with VAR Requirements 9 (injection into the soil within eight hours) or 10 (incorporate into the soil within eight hours), and have a federally acceptable Class A and vector attraction processing scheme. The PEC has not been approached yet on this potential process.

Lime treatment processes. Lime and other bases are very effective in destroying viruses and pathogenic bacteria, provided pH is brought to 12 and maintained for a sufficient time. They are not effective in destroying helminth eggs. Class A reductions are achieved by raising the temperature to meet the thermal equation of Alternative 1, using either extra lime addition to release heat and increase the temperature, or by adding external heat. There are a number of possibilities for improving the destruction of helminth eggs that do not require addition of extra base or external heat. They do require operating on sludge before it is dewatered. One possibility is simply applying high pressure, about 700 psi (Buitron et al. 1998). Applying pressure to an incompressible liquid is not a costly operation. Another possibility is to use ultrasonic force. This is not effective for reducing bacteria or viruses, but it could be effective for the much larger helminth eggs.

Long-term storage. Long-term storage of sludge in lagoons (about two years) was demonstrated as an effective way to eliminate pathogens in warm climates several years ago. A Water Environment Research Foundation project (No. 95-REM-2, "Obtaining Pathogen Equivalency Certification for Class A Biosolids through Storage" and "Air Drying Research Program to Establish Practical Temperature Range of the Process of Lagoon Storage of Biosolids for Eliminating Pathogens"—estimated completion date October, 2004) is being carried out (personal communication, P. Schafer) to define more closely the time required. The City of Chicago uses this technique to produce a Class A sludge. At Chicago, the sludge is stored for a long period in lagoons, and is then air-dried to about 60 percent solids. The process has not received PEC approval as yet. At present, the material is checked in batches. This is an application of the Class A Alternative 4. Ahmed and Sorensen (1993) have stored sludge cake in piles for long periods and have shown great declines in pathogen densities. Satisfactory proof that storage as cake in piles produces an adequate reduction in pathogens is difficult to demonstrate, because the interior and exterior of the pile are subject to such different conditions. The task is much easier for lagoons where conditions are uniform. These approaches are very interesting because they have worldwide application and they use unsophisticated technology.

Electron beam and gamma ray irradiation. These processes are listed in the regulation as PFRPs. They are effective but to this writer's knowledge, they are not being used. An electron beam facility was built and still exists at Miami, FL, but is not in use. Sandia Laboratories built a pilot-scale gamma ray irradiation unit in Albuquerque, New Mexico but this unit is probably dismantled. The gamma ray facility was planned for use with ^{137}cesium, but this material has never become available. Part of the reason for lack of

enthusiasm for using gamma rays is risk of escape of a long-lived radioactive material. The solubility of salts of cesium increases that concern. ^{60}Cobalt could be used for gamma ray irradiation but costs would be higher. The electron beam has less potential for accident (positive shut-off, low penetration). The low penetration power is a disadvantage in that the sludge must be presented to the electron beam in a thin film. The dose needed in these processes is high, about one megarad for destruction of viruses, the most resistant organisms. Costs are directly proportional to dose. However, helminth eggs are destroyed with a dose of under 0.1 megarad (Stern and Farrell 1978). Combining a high-energy radiation process with another process that destroys viruses and bacteria has potential.

Other processes. There are a number of processes under development that may have potential for achieving Class A. Some of these are: vermicomposting under rigidly controlled conditions (Eastman, 2000), use of microwave technology to provide rapid heating to pasteurization temperatures, and some variations of lime treatment, which could include the addition of other chemicals.

Research And Process Evaluation Needs

- Conduct methods development research. We have been uncertain about the best method to determine *Salmonella* spp. since the regulation was published. Practitioners can choose among three methods referenced in the regulation, two of which are probably inadequate. Some people may choose the method that is least likely to show a positive reading.

- A process is needed to evaluate the perfomance of the various laboratories that are doing the analyses. Possibly a requirement to send a spiked sample periodically would reveal careless, incompetent or dishonest work.

- Develop a procedure for quantifying hepatitis A virus in sludge. Determine reductions with conventional processing such as anaerobic digestion and compare it to reductions in enteroviruses. Investigate some of the bacterial pathogens in the same way. Compare reductions to reductions in fecal indicators and salmonellae.

- Require applicants for new E-PSRPSs and E-PFRPs to spell out their processes in sufficient detail. Do the same for existing PFRPs and PSRPs. For example, we need to say more about anaerobic digestion than that it should be run at 35°C with a nominal residence time of 15 days or greater. Regulators and operators need adequate guidance to assure that processes are operating as intended. As noted above, some processes are basically simple and need minimal control, whereas others are affected by known and sometimes unknown variables. The regulator needs to be alerted to potential trouble spots so that he or she can see if records and operating controls are adequate.

- The resistance of helminths to inactivation has long been known, yet fundamental work on the nature of the destruction process has not been done. The approach of practitioners and process developers has been empirical. For example, we think that ammonia penetrates the eggs and inactivates them, but we have not looked deeply into the effect of concentrations, temperature, nature of the sludge or wastewater that contains them, and the stage of larval development. Research to investigate such effects is long overdue.

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Pathogens in Biosolids: Risks and Regulations

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Introduction

This workshop provides us with an opportunity to synthesize our current knowledge about pathogens in biosolids and manures and to focus our attention on issues that need to be addressed in order to ensure a safe and sustainable future for biosolids reuse. This paper focuses on the risks posed by pathogens in biosolids and the guidelines that have arisen in Australia and the US to manage those risks. Ideally, the development of guidelines follows on from a thorough understanding of risk. However, the need for guidelines often precedes our ability to measure risks and in the absence of that information, guidelines are developed in other ways, often on the basis of technical feasibility. It is important that we continue to review our guidelines in the light of improved understanding of risks.

This paper highlights some of the differences between biosolids guidelines in Australia and the US. It also summarizes our current understanding of the health risks posed by exposure to biosolids. I hope to stimulate thought on appropriate ways to determine acceptable pathogen levels for the purposes of guidelines, as well as appropriate ways to monitor these levels and improve our understanding of risk.

Australian Biosolids Guidelines

To date, no national guidelines to govern the reuse of biosolids in Australia have been agreed upon, although there have been guidelines in draft form for almost a decade (e.g., ARMCANZ/ANZECC, 2002). When these guidelines are introduced, it is intended that they will be complemented by separate state guidelines that take into account local conditions. However, in the absence of national guidelines, some Australian states have already begun developing their own guidelines. New South Wales is the only state to have implemented guidelines (NSW EPA, 1997) so far. It is quite likely that these will form the basis of guidelines in other states.

The Australian draft national guidelines and the New South Wales guidelines are similar to US regulations in many ways. Both are based on pathogen reduction processes and the use of barriers to prevent human or animal contact. For end uses where some degree of human contact is likely, additional microbial limits are set. Thus, like US regulations, Australian guidelines classify biosolids and allow certain end uses based on the stabilization process used. Approved stabilization processes are similar to those specified in US regulations (U.S.EPA 1992a) and are summarized in Appendix 1. For biosolids that are available for unrestricted use, there is an additional requirement to verify the effectiveness of the treatment process by determining pathogen and indicator levels.

It is this last point, the pathogen and indicator levels, where differences arise between the guidelines of the two countries. As shown in Table 1, different organisms are used to indicate treatment efficiency, both between Australia and the US and within Australia itself. There are also differences in the acceptable levels of these organisms.

One notable difference is the use of helminth ova as indicators of the effectiveness of treatment. US EPA regulations include helminths while draft Australian guidelines do not. New South Wales guidelines do include helminths but only as an initial verification that treatment processes conform to specified standards. This difference may reflect the low incidence of helminth infections in urban Australian communi-

Table 1. Acceptable Pathogen and Indicator Concentrations in Biosolids Suitable for Unrestricted Use

Pathogen / Indicator	US EPA (US EPA, 1992a)	Australian Guidelines (draft) (ARMCANZ/ ANZECC, 2002)	New South Wales Guidelines (NSW EPA, 1997)
Salmonella spp.	< 3MPN/4 g total dry solids	< 1MPN /50g final product	Not detected /50 g dry weight
Helminth ova	<1 viable ova / 4 g total dry solids	Not specified	< 1 /4g total dry solids*
Viruses	< 1 PFU /4 g total dry solids	Not specified	< 1 PFU /4g total dry solids*
Fecal coliforms	< 1000 MPN / g total dry solids	< 100 /g final product	< 1000 MPN /g dry weight
E. coli	Not specified	Not specified	< 100 MPN/g dry weight

* Monitoring required for initial process verification only. Not included in routine monitoring

ties. Table 2 shows the prevalence of some enteric pathogens in urban and nonurban Western Australia. These data are presented separately because in Australia the prevalence of communicable diseases is generally higher in nonurban areas than in urban areas. Biosolids from nonurban areas are rarely reused in Western Australia, where there is either no centralized wastewater treatment or where the volume of biosolids is so small that it is simply stored. It is expected that the situation would be similar in other areas of Australia.

As can be seen from Table 2, the prevalence of helminth infections in metropolitan Western Australia is low and, correspondingly, the density of helminth ova in biosolids would be expected to be low. Gibbs et al. (1995) surveyed raw sludge and anaerobically digested and dewatered biosolids from three wastewater treatment plants in Perth, Western Australia and failed to positively identify any helminth ova. Ova were found in two of 18 (11%) raw sludge samples but could not be positively identified as hookworm ova. It is possible that these were eggs of soil nematodes. Thus, for some Australian situations, lack of helminth ova in biosolids doesn't necessarily indicate effective pathogen reduction. However, given that the prevalence of helminth infections is likely to vary throughout the country it may be reasonable to include a requirement for helminth reductions in broad, national guidelines.

As shown in Table 1, the concentration of salmonellae that is considered to indicate effective pathogen reduction also differs between Australia and the US. Under US regulations, Class A biosolids should have less than 3MPN /4 g dry solids while under draft Australian guidelines it is proposed that salmonellae should be less than 1 MPN/ 50 g final product. The US limit is based on the detection limit of the analytical method. The differences between these limits are further discussed in the following section.

Thus Australian guidelines have largely been based on the health and environmental risk assessment methodology developed by the USEPA (ARMCANZ/ANZECC, 2002), with some adaptations for local conditions. Guidelines are expected to be modified as research is conducted under Australian conditions (ARMCANZ/ANZECC, 2002).

Acceptable Pathogen Concentrations

An important consideration in the development of biosolids guidelines is the level of pathogens and indicators that will be considered acceptable. As discussed above, there are differences between these levels in US and Australian guidelines.

The traditional approach to monitoring microbiological quality is, of course, to monitor indicator organisms, typically fecal coliforms and fecal streptococci. In some cases, it has also been considered feasible and necessary to monitor pathogens directly. US regulations require monitoring of salmonellae in composted biosolids and it is likely that Australian guidelines will develop along similar lines. (*Editor's Note*: All of the Class A alternatives under the US regulation allow the direct measurement of *Salmonella* spp. or fecal coliform analysis but do not require both. See U.S.EPA 1999.)

Table 2. Prevalence of Selected Enteric Pathogens in Western Australia, 1991

Pathogen	Perth Metropolitan Area		Kimberley Region(tropical, nonurban area)	
	No. cases	**Prevalence (%)**	**No. cases**	**Prevalence (%)**
Salmonella spp	376	0.034	167	0.712
Giardia	514	0.032	75	0.320
Hookworms	26	0.002	96	0.409
Ascaris	12	0.001	0	0
Taenia saginata	1	<0.001	0	0
Hymenolepis nana	25	0.002	95	0.405

(Health Department of Western Australia, 1992)

To monitor for pathogens in biosolids suggests that there is some concentration of pathogen that is acceptable. Likewise to monitor indicator levels suggests that treatment has effectively reduced pathogens to an acceptable concentration. Experience with the 1998 Sydney Water Crisis clearly demonstrates the dangers of monitoring for pathogens when we do not have a full understanding of the risk they pose. In this incident, *Cryptosporidium* and *Giardia* were detected in Sydney's water supply and boil water alerts were issued on three separate occasions. Some of the events that followed included an extensive inquiry, the creation of a new catchment management authority and lawsuits. However, no additional cases of illness were observed and some people now suggest that the entire crisis was the result of laboratory error. (For further information see Clancy (2000a or 2000b) and Hawkins (2000)). This was a case of having to base management decisions on data when it was not clear how the data should be interpreted. If we are going to monitor for pathogens (or indicators) in biosolids and sensibly interpret those results, we must have a clear understanding of the basis for any limit.

Acceptable limits for pathogens in biosolids are frequently derived on the basis of the detection limits of the analytical methods used. For example, US limits are based on the lower detection limits of the methods used to analyze for the pathogens. The US EPA (1992) stated that "The implicit goal of the Class A requirements is to reduce the pathogens in sewage sludge.... to below detectable limits". This approach to setting acceptable limits presents two problems:

1. Detection limits change as methods are improved and new techniques are developed.

2. The detection limit of an assay may have no biological relevance. Using a detection limit to set a regulatory limit may lead to a stricter limit than required to protect public health, thereby placing unnecessary burdens on the biosolids industry. Alternatively this practice may lead to a limit that is not protective of public health. Rather, it would lead the public to assume that exposure to a product that met regulatory requirements was safe.

An alternative method of deriving acceptable limits is to use epidemiological evidence. Evidence from a series of epidemiological studies such as those by Cabelli et al. (1982) and Kay et al. (1994) was used to set acceptable limits in bathing water quality guidelines. Of course, this approach is not without its problems, which arise because of the uncertainties of epidemiological studies. Mugglestone et al. (2000) highlighted some of the problems with this approach in relation to WHO bathing water quality guidelines. However, with rigorously designed studies most of these problems can be overcome.

At present there appears to be insufficient epidemiological data related to biosolids to enable limits to be set on this basis. Early epidemiological studies of biosolids exposure such as those by Lundholm and Rylander (1980) and Clark et al. (1984) failed to produce conclusive results because of small or otherwise limited sample populations or because they did not control for confounding factors. As will be discussed later, we conducted an epidemiological study of health risks associated with exposure to composted biosolids products. This study was not designed to determine any correlation between indicator organisms or pathogens and illness so the results of this study could not be used to set acceptable limits. However, given that this approach has been used successfully for bathing waters, it would appear that it is within the capabilities of currently available techniques to generate the necessary information.

Another approach to setting acceptable limits is to use a modification of the quantitative risk assessment process. This would involve determining an acceptable limit based on an acceptable risk. Simmonds et al. (1998) suggested that acceptable risk could be defined on the basis of the following three principles:

- *Principle 1: Risk assessments for microbial pathogens in biosolids should aim to protect the most at risk individual.* The most at risk individual is the person who is most exposed to the pathogens in the biosolids. In the case of salmonellae and other enteric pathogens this will be the person who is both most likely to ingest the biosolids and who is most susceptible to infection.

- *Principle 2: Microbial risk assessments should be based on risks of disease rather than the risks of death.* Because the diseases caused by enteric pathogens in biosolids are rarely life threatening, microbial risks were evaluated in terms of disease rather than death.

- *Principle 3: The risk of disease transmission through the reuse of biosolids products should be less than background transmission rates for that disease from all other sources.* Defining acceptable risk is a controversial process but it is suggested that it could be between 1 and 10% of the background rate of infection.

Based on the assumption that children were the most at risk because of their tendency to ingest soil (and it was assumed that children with pica behavior would ingest 5g of soil), Australian data for levels of salmonellae infections in the community (Anura and Hall 1992) and the dose response model described by Rose and Gerba (1991), Simmonds et al. (1998) calculated an acceptable limit of between 1 salmonellae in 30 g and 1 salmonellae in 300 g. They recommended a limit of 1 salmonellae in 50 g partly in recognition of practical limitations and because it represented a probability of infection which was 6% of the reported Australian background rate of infection.

One of the key issues, then, of developing guidelines and regulations, whether for indicators or pathogens themselves, is how should acceptable pathogen or indicator levels be derived? While detection limits have served this purpose in the past, other approaches may now warrant consideration.

Health Risks of Exposure to Biosolids

Clearly one of the major goals of biosolids management remains an improved understanding of the health risks posed by pathogens in biosolids. A thorough understanding is perhaps some way off, but our knowledge in this area has increased in recent years.

Quantitative risk assessment and epidemiology are the two main techniques that have been used in an attempt to understand the risks posed by pathogens in biosolids. Although these techniques are complementary, they are quite distinct in their requirements and outcomes.

The use of quantitative risk assessment in environmental microbiology has increased considerably in recent years. The processes of hazard identification, dose-response exposure assessment and risk characterization are familiar techniques. The use of quantitative risk assessment in relation to biosolids is discussed in the Eisenberg et.al. and P. Gale papers.

The use of epidemiology with respect to biosolids has been fraught with difficulties. This is principally because only a small number of people exposed are expected to become infected and an even smaller number show signs of disease. Further difficulties arise because a wide range of clinical manifestations can be associated with one type of pathogen or, conversely, several pathogens can cause similar symptoms. Thus, to be statistically valid, large studies are needed and it is difficult to pinpoint the cause of any symptoms. The US EPA sponsored 11 epidemiological studies related to wastewater and biosolids and none were able to provide conclusive evidence of increased disease risks (Jakubowski 1986).

There is, however, evidence that pathogens can be transmitted to humans from potting mixes and other gardening products similar to those made from composted biosolids. In Western Australia, infections with *Legionella longbeachae* are strongly associated with exposure to gardening and potting mixes (Brennan 1995; Gabbay et al. 1996). There is no evidence to suggest that biosolids are the source of *L. longbeachae* but this does suggest that exposure to pathogens in products such as potting mixes can lead to infection.

Biosolids Epidemiological Research in Australia

In an attempt to better quantify health risks posed by biosolids, we undertook a prospective cohort study to determine the incidence of gastrointestinal and respiratory illness in people exposed to compos-

ted biosolids in gardening products (Simmonds et al. 1999). The incidence of these illnesses was compared with that in a group of people who used similar products made without biosolids. Approximately 1,700 people took part in the study. We found no significant association between exposure to composted biosolids and the incidence of gastrointestinal illness. However, there was a significant association between exposure to biosolids and respiratory illness. Adults exposed to composted biosolids had a relative risk (95% confidence interval) of respiratory illness of 4.13 (1.38, 12.42). In other words, they were four times more likely to suffer respiratory illness after using composted biosolids products than those who used products without composted biosolids. The cause of the illness observed in our study is not known but the growing body of evidence that associates swimming in sewage-contaminated waters with respiratory illness strengthens the plausibility of this finding (see, for example, D'Alessio et al. 1981; Cabelli et al. 1982; Kay et al. 1994; McBride et al. 1998).

The implications of this research need to be interpreted in terms of acceptable risk, a controversial subject. Although there was no observed increase in gastrointestinal illness associated with exposure to biosolids in our study, it was not possible to detect low levels of risk. The study was designed to detect a doubling of the incidence of disease with 80% power. Thus if we take the results of this study to mean that there was no increase in risk of gastrointestinal illness, we are effectively accepting up to a doubling of the background incidence of disease. If the currently measured level of risk is not considered acceptable, further work will be needed to measure the risks of gastrointestinal illness more precisely. As a very large study would be needed to do this using epidemiological techniques, it is suggested that quantitative risk assessment could be used in the future.

Further development of quantitative risk assessments would benefit from being validated against epidemiological information. We compared the results of our epidemiological study with the results of a trial quantitative risk assessment model produced by the USEPA (1991a, 1991b, 1992b) (Simmonds et al. 2000). Although the comparison was limited in a number of ways, the results of the two methods showed general agreement for bacterial and parasitic pathogens but suggested that the quantitative risk assessment model underestimated the risks posed by viruses. This may have been due to the fact that the concentration of viruses in biosolids assumed in the risk assessment model was probably lower than that found in Perth biosolids (Gibbs et al. 1995).

Future epidemiological studies would benefit from an improved study design. The gold standard format of any epidemiological study is a randomized control trial. In such a study, participants are randomly divided into (usually) two groups, one of which is exposed to the factor of interest and the other of which is a control. This type of study design provides the greatest evidence for concluding causation because many types of bias or confounding that hamper other study designs are controlled by being evenly distributed between the groups. Randomized control trials are increasingly being used in environmental epidemiological studies. For example, this type of study was used to study the health effects of swimming in sewage-contaminated seawater (Kay et al. 1994) and the health effects of consuming tap water (Payment et al. 1991, 1997).

The implications of these findings for biosolids management are uncertain because there is little information about the causative agents of respiratory illness (assumed to be enteroviruses and adenoviruses) in composted biosolids. Little is known about the concentrations or inactivation of viruses in composted biosolids. It is also uncertain from our epidemiological study whether the route of transmission of the etiologic agent to users of composted biosolids products was via ingestion or inhalation. It is therefore difficult to know if measures such as modifying biosolids treatment or the way in which products are handled would reduce the risk of respiratory illness. Further research is needed to identify the etiologic agents of respiratory illness, to improve detection methods for such agents in biosolids, and to improve the understanding of the role biosolids may play in transmission of these agents.

Conclusions

Quantifying the risks posed by pathogens in biosolids remains one of the biggest challenges for biosolids management. There is the need for continued development of both quantitative risk assessment and epidemiological data and this requires the improvement of pathogen detection methods. As viruses or other etiologic agents may have been responsible for an increase in respiratory illness observed in those exposed to composted biosolids products, identification of those agents and development of methods for their detection in biosolids are areas that deserves attention.

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Appendix 1. Summary of Approved Biosolids Stabilisation Processes

Draft Australian guidelines (ARMCANZ/ANZECC, 2002)	NSW EPA guidelines (NSW EPA, 1997)
Pathogen Grade P1 (Unrestricted use) Composting in-vessel method: Temperature of all the compost material is to be maintained at > 55°C for 3 continuous days. Biosolids to be digested prior to composting. 30 days maturation of product required before use Composting Windrow method: Temperature of all the compost material is to be maintained at > 53°C for 5 continuous days. Minimum of 5 turnings of windrow. 30 days maturation of product required before use pH and heating: pH of the biosolids product to be raised above 12 and pH to remain> 12 for a minimum of 72 continuous hours. During this 72 h period the temperature must be >52°C. At the end of the 72 h period, biosolids product shall be air dried to final solids content >50%. Undigested sludge must be aerated in a windrow for 15 days with a minimum of five turnings or demonstrate minimum regrowth potential Heating and drying: Biosolids are dried by heating particles to > 80°C to achieve a final solids content of at least 90% w/w. Final product to be kept dry until applied. Product from undigested sludge to demonstrate minimum regrowth potential Long term storage: Sludge is digested, dewatered to a solids content > 10%, stored under aerobic conditions for > 3 years. Product must be stored to prevent contamination Other processes: Must demonstrate 100% Taenia or Ascaris (seeded) egg inactivation and < 1 enteric virus/100 g final product Grade P2 (Landscaping other than household application, root crops, pasture/fodder for dairy cattle) Composting: Temperature of all compost to be maintained >53°C for 5 continuous days or > 55°C for 3 continuous days Heating and drying: Biosolids heated to >70°C and dried to a solids content >75%. Undigested sludge shall be dried to at least 90% dry solids w/w. Final product to be kept dry until applied Aerobic thermophilic digestion: Maintain aerobic conditions at a temperature of 55°C to 60°C for 10 continuous days. Volatile solids reductions > 38%. Product dried to final solids content >50% Other Processes: Must provide equivalent reduction of pathogens, volatile solids, odours Grade P3 (Cooked or processed crops, pasture/fodder for cattle, forestry, land rehabilitation, municipal landfill) Anaerobic digestion: 15 days at 35°C or 60 days at 15°C. > 38% volatile solids reduction. <2 000 000 MPN Thermotolerant coliforms per gram (dry weight) Aerobic digestion: 40 days at 20°C or 60 days at 15°C. > 38% volatile solids reduction. <2 000 000 MPN Thermotolerant coliforms per gram (dry weight) Composting: Aerobic conditions maintained. 5 days >40°C including 4 hours at > 55°C. <2 000 000 MPN Thermotolerant coliforms per gram (dry weight) Grade P4 (Secure landfill or thermal processing) Any stabilisation process not meeting above conditions	Stabilisation Grade A (Unrestricted use) Thermal treatment: Includes composting at 55°C for 3 days, pasteurisation, hot gas stream in a rotary drier, thermophilic anaerobic digestion High pH – high temperature: pH of the biosolids product is to be raised to greater than or equal to pH 12 and remain above 12 for 72h. During at least 12 h of the 72 h period, the temperature of the biosolids has to be > 52°C. After 72 h the biosolids product must be air dried to a solids content of > 50% Other processes: Includes biosolids that have been dewatered and stored for > 3 years. Must demonstrate < 1 PFU/ 4g viruses and < 1/4 g helminth ova Stabilisation Grade B (Agriculture, forestry, rehabilitation, landfill, surface land disposal within sewage treatment plant site) Anaerobic digestion Aerobic digestion Air drying Composting Lime stabilisation Extended aeration Other approved processes Stabilisation Grade C (Landfill or surface land disposal within sewage treatment plant site) Not meeting any of the above requirements

Regulations and Strategies for Controlling Pathogens in Biosolids in the UK

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United Kingdom

Background

Following reorganization of the UK water industry in 1974, from numerous local authorities into a small number of large regional water authorities, the newly constituted National Water Council (NWC) investigated the implications of the microbiological contamination of sewage sludges used in agriculture. Based on unpublished reports of salmonellosis in livestock animals, NWC concluded that salmonellas constituted the greatest risk of infection to livestock. In 1981 the European office of the World Health Organization (WHO, 1981) published a review of the health risks arising from the application of sewage sludge to land. This review recognized that the risks to human health from salmonellas and beef tapeworm were greater than for other pathogens considered. During the early 1980s, DGXII of the European Commission instituted a Co-operative Action program (COST 68, 1982) on the treatment and disposal of sewage sludge, the results of which influenced the development of the EC Directive (86/278/EEC). (*Editors Note*: The European Commission is divided into a number of Directorates General — DG — to cover economic, tourism, agriculture, and other areas. DG XII is responsible for Environment. COST is an acronym derived from European Cooperation in the field of Scientific and Technical research.)

The controls on the application of sewage sludge to agricultural land within member states of the European Union (EU) derive from Council Directive 86/278/EEC published in 1986 for implementation within three years (CEC, 1986). The principal rationale of the Directive was to minimize the accumulation in the soil of heavy metals or other potential toxic elements with the objective of protecting soil fertility and public health. However, the Directive also included measures for controlling transmissible disease by introducing constraints on the use of sludge. Article 7 of the Directive requires Member States to prohibit the use of sludge or the supply of sludge for use on:

a) grassland or forage crops if the grassland is to be grazed or the forage crops to be harvested before a certain period has elapsed. This period, which shall be set by the Member States taking particular account of their geographical and climatic situation, shall under no circumstances be less than three weeks;

b) soil in which fruit and vegetables are growing, with the exception of fruit trees;

c) ground intended for the cultivation of fruit and vegetable crops which are normally in direct contact with the soil and normally eaten raw, for a period of 10 months preceding the harvest of crops and during the harvest itself.

In addition, the Directive required that sludge shall be treated before being used in agriculture. (Treated sludge is defined in Article 2(b) of the Directive as "sludge which has undergone biological, chemical or heat treatment, long term storage or any other appropriate process so as significantly to reduce its fermentability and other health hazards resulting from its use".) Member States may nevertheless authorize, under conditions laid down by them, the use of untreated sludge if it is injected or worked into the soil.

In the UK, The Sludge (Use in Agriculture) Regulations 1989 directly implement the provisions of the Directive (Anon, 1989). This was accompanied by a Code of Practice (DoE, 1989, 1996), which provided practical guidance on how the requirements of the Directive could be met. It recognizes that pathogens may be present in untreated sludges and that their numbers can be reduced significantly by appropriate treatment. Examples of effective treatment processes are given in the Code (Table 1). At the time that the Code was prepared the pathogens of concern were considered to be salmonellas, *Taenia saginata* (human beef tapeworm), potato cyst nematodes (*Globodera pallida* and *Globodera rostochiensis*) and viruses.

The guidance given in the UK DoE Code of Practice was based on the concept of multiple barriers to the prevention of transmission of pathogens when sludge was applied to agricultural land. The barriers are:

- Sludge treatment, which will reduce pathogen content
- Restrictions on which crops may be grown on land to which sludge has been applied
- Minimum intervals before grazing or harvesting (Table 2)

The scientific and public health principles which underpin this concept are valid. They recognize that for certain crops the risk of disease transmission is unacceptable, e.g., salad items which have a short growing period and which are to be consumed raw. For other crops, the combination of treatment and a suitable period of no harvesting will result in the numbers of pathogenic microorganisms being reduced below a minimum infective level. Despite the current concerns surrounding the risks to food safety, it is important to recognize that there have been no instances documented in which disease transmission to man or animals has occurred where the provisions of the relevant UK Regulations and Codes of Practice were followed (RCEP, 1996). More recently a review of the scientific basis to the existing controls suggested that there is a potential risk (though extremely small) of disease transmission from sewage sludge to grazing animals and that only treated sludge should be applied to the surface of grassland and land used for growing food crops (WRc, 1998). There is no evidence in the UK that human health has been put at risk through the transfer of pathogens from sewage sludge – it is more likely that pathogens could be transferred through poor food handling practices and use of untreated farm wastes. The annual amount of sludge produced in the UK is in the region of 750,000 tonnes (1000 kg) dry solids (tds), of which about 50% is applied to agricultural land.

Table 1. Examples of Effective Sludge Treatment Processes as Defined in the UK Code of Practice (DoE, 1989)

Process	Conditions
Pasteurization	Minimum 30 min at 70°C; or Minimum 4 hr at 55°C Followed in all cases by mesophilic anaerobic digestion
Mesophilic anaerobic digestion	Mean retention of at least 12 d at 35°C $\pm$ 3°C; or Mean retention of at least 20 d at 25°C $\pm$ 3°C. Followed in each case by secondary digestion with a mean retention period of at least 14 d.
Thermophilic aerobic digestion	Mean retention of at least 7 d. All sludge to be subjected to a minimum of 55°C for at least 4 hr.
Composting (windrows or aerated piles)	Compost must be retained at 40°C for at least 5 d including a period of 4 hr at a minimum of 55°C. Followed by a period of maturation.
Alkaline stabilization (with lime)	pH to be 12 or greater for a period of at least 2 hr.
Liquid storage	Storage for at least 3 months.
Dewatering and storage	Dewatering and storage for at least 3 months. Storage at least 14 d if sludge previously subjected to mesophilic anaerobic digestion.

Source DoE, 1989, 1996

Recent Developments

BSE

Bovine Spongiform Encephalopathy (BSE), sometimes referred to as mad cow disease, a progressive, fatal disease of the nervous system of cattle was first identified in the United Kingdom in 1986 fol-

Table 2. Acceptable Use of Untreated and Treated Sludge in Agriculture (from DoE, 1989)

Crops	Restrictions when applied PRIOR to planting	Restrictions when applied AFTER planting
Treated Sludges		
Cereals	None	None
Oil seed rape	None	None
Grass	None	No grazing for 3 weeks
Fodder, sugar beet, etc.	None	Not Permitted
Turf	N/A	>3 months
Fruit trees	None	>3 months
Soft fruit	>3 months	Not Permitted
Vegetables[1]	>3 months	Not Permitted
Potatoes[2]	>3 months	Not Permitted
Nursery stock	Not Permitted	Not Permitted
Untreated Sludges[3]		
Grass	None	No grazing for 3 weeks
Turf	N/A	>6 months
Cereals, grass, fodder, sugar beet, oil seed rape, etc.	None	Not Permitted
Soft fruit, Vegetables	>10 months	Not Permitted
Potatoes[2]	>10 months	Not Permitted

1. For crops normally in direct contact with soil and may be eaten raw
2. Not to be applied to land used or to be used for a cropping rotation that includes seed potatoes
3. Untreated sludges must be cultivated or injected into the soil
N/A Not Applicable

lowing a case in 1985. BSE belongs to a group of diseases known as transmissible spongiform encephalopathies (TSE). There have been 178,639 cases of confirmed BSE in UK cattle up to 14 June 2002 (http://www.defra.gov.gov.uk/animalh/bse/index.html). The primary control measure to prevent transmission was the ruminant feed ban, introduced in 1988. Later, the Government has introduced and strengthened controls to reduce the risk of people eating beef and meat products that might contain the BSE agent. Measures to reduce the risk to human health are the Over Thirty Month (OTM) rule and the Specified Risk Material (SRM) Order. The OTM rule bans cattle older than 30 months from sale as food for humans. It is based on knowledge that BSE very rarely presents in cattle less than 30 months and that even if they were infected the amounts of infectious agent would be small. The SRM order requires that the parts of cattle and sheep most likely to carry BSE (brain tissue, spinal cord) must be removed. Since 1996, controls have been in place to prevent solids larger than 0.1 g entering the liquid waste discharged to the sewerage system. All SRM is removed and rendered. Rendering inactivates at least 98% of the BSE infectivity (Taylor et al., 1995). The risk of transmission of BSE to cattle and vCJD to humans as a result of applying sewage sludge to agricultural land has been modeled by Gale and Stanfield (2001). They calculate that the annual risk of BSE infection to a single animal is 5.4×10^{-5}. The exposure to humans via the consumption of vegetables grown on soil receiving sewage sludge produced an annual risk of variant Creutzfeldt-Jakob disease (vCJD) of 1.0×10^{-7} person^{-1}.

The Safe Sludge Matrix

Against a background of concern over food production methods, the water industry in the UK agreed to a set of guidelines matching the level of sewage treatment with the crop under cultivation. This agreement was made under the auspices of Water UK, and representatives of the food suppliers.

The Safe Sludge Matrix (http://www.adas.co.uk/matrix/; Table 3a,b) forms the basis of the agreement. It consists of a table of crop types, together with clear guidance on the minimum acceptable level of treatment for any sewage sludge based product, which may be applied to that crop or rotation. The agreement was driven by the desire to ensure the highest possible standards of food safety and to provide a frame-

Table 3a. Safe Sludge Matrix

Crop Group	Untreated Sludges	Conventionally Treated Sludges	Enhanced Treated Sludges
Fruit	✗	✗	✓ (10 month harvest interval applies)
Salads	✗	✗ (30 month harvest interval applies)	✓ (10 month harvest interval applies)
Vegetables	✗	✗ (12 month harvest interval applies)	✓ (10 month harvest interval applies)
Horticulture	✗	✗	✓ (10 month harvest interval applies)
Combinable & Animal Feed Crops	✗	✓	✓
Grass & Forage - Grazed	✗	✗ (Deep injected or ploughed down only) (3 week no grazing and harvest interval applies)	✓ (3 week no grazing and harvest interval applies)
Grass & Forage - Harvested	✗	✓ (No grazing in season of application) (3 week no grazing and harvest interval applies)	✓ (3 week no grazing and harvest interval applies)

Note: ✓ All applications must comply with the Sludge (Use in Agriculture) Regulations and DETR Code of Practice for Agriculture Use of Sewage (to be revised during 2001).

✗ Applications not allowed (except where stated conditions apply)

work that gives the retailers and food industry confidence that sludge reuse on agricultural land is safe. The Matrix enables farmers and growers to continue to utilize the beneficial properties in sewage sludge as a valuable and cost-effective source of nutrients and organic matter.

The main impact of the Safe Sludge Matrix was the cessation of raw or untreated sewage sludge being used on agricultural land. From the end of 1999, all untreated sludges have been banned from application to agricultural land used to grow food crops. The Matrix introduces the concept of two classes of treatment — analogous to the US 503 Regulations (USEPA, 1993) —*Treated* and *Enhanced Treated* (Table 4). Treated sludge can only be applied to grazed grassland where it is deep injected into the soil. The regulations require that there will be no grazing or harvesting within three weeks of application. Where grassland is reseeded, sludge must be ploughed down or deep injected into the soil. More stringent requirements apply where sludge is applied to land growing vegetable crops and in particular those crops that may be eaten raw (e.g., salad crops).

Treated sludge can be applied to agricultural land that is used to grow vegetables provided that at least 12 months have elapsed between application and harvest of the following vegetable crop. Where the crop is a salad, which might be eaten raw, the harvest interval must be at least 30 months. Where enhanced treated sludges are used, a 10-month harvest interval applies.

The Department of the Environment, Transport and the Regions (DETR) has announced that it intends revising the Regulations and Code of Practice to take account of the Safe Sludge Matrix. It is envisaged that the revised regulations and code of practice will be introduced into parliament during 2001 following a consultation process. It is proposed to establish process monitoring based on the principles of HACCP (Hazard Analysis and Critical Control Point) allied to an end product standard (Table 4). The rationale behind this approach is to establish the critical control points within the sludge treatment process to assure pathogen reduction. Wherever possible, monitoring will be carried out to demonstrate that the process is operating within the control limits set. Microbiological analysis of the final treated sludge serves to verify

Table 3b. Cropping Categories Within the Safe Sludge Matrix

Fruit	Salad (e.g. ready to eat crops)	Vegetables	Horticulture	Combinable and animal feed crops	Grassland and forage	
					Harvested	Grazed
Top fruit (apples, pears, etc.) Stone fruit (plums, cherries etc.) Soft fruit (currants and berries) Vines Hops Nuts	Lettuce Radish Onions Beans (including runner, road and dwarf French) Vining peas Manetout Cabbage Cauliflower Calabrese/broccoli Courgettes Celery Red beet Carrots Herbs Asparagus Garlic Shallot Spinach Chicory Celeriac	Potatoes Leeks Sweetcorn Brussel sprouts Parsnips Swedes/turnips Marrows Pumpkins Squashes Rhubarb Artichokes	Soil based glasshouse and polythene tunnel production (including tomatoes, cucumbers, peppers etc.) Mushrooms Nursery stock and bulbs for export Basic nursery stock Seed potatoes for export Basic seed potatoes Basic seed production	Wheat Barley Oats Rye Triticale Field peas Field beans Linseed/flax Oilseed rape Sugar beet Sunflower Borage	Maize silage Grass silage Haylage Hay Herbage seeds	Grass Forage Swedes/turnips Fodder mangolds/ beet/kale Forage rye and Triticale Turf production

Table 4. Proposed UK Standards for Treated and Enhanced Treated Sludge

Sludge Category	Reduction in numbers of E. coli across sludge treatment process	Number of E. coli in final product	Number of salmonellae in final product
Treated	2 Log_{10}	10^5 g^{-1} ds(1)	No standard
Enhanced Treated	6 Log_{10}	10^3 g^{-1} ds(2)	Absent from 2g ds(3)

that the controls are effective. The sampling frequency necessary for verification is significantly below that required if product quality were assessed solely on the basis of microbiological analysis.

The question which must be addressed is how to relate log reductions of *E. coli*, an indicator organism, to pathogen destruction. The approach being adopted relies heavily on a risk assessment framework which links the elements of the major UK program of research which is entering its final stages (see *Reduction in Pathogens During SludgeTreatment* below). The intention is to establish the capability of the major sludge treatment processes to inactivate pathogens (bacteria, virus, and protozoan parasites) and the indicator organism *E. coli*. The data from the experiments will be used to calculate net reduction rates for each of the pathogens investigated which will be fed into the risk assessment model (see Chapter by Gale).

1. To be achieved on 90% basis for first 2 years following introduction of the Regulations with a maximum allowable concentration of 10^7 g^{-1} dry solids (ds); thereafter maximum allowable concentration of 10^5 g^{-1} ds.

2. It is expected that well controlled sludge treatment is likely to consistently produce a sludge containing less than 10^2 g^{-1} ds. Should routine sampling indicate that the product quality was poorer than this, then the operation of the plant must be checked, even though the batch quality was within the final product standard.

3. In five random samples each containing 2 g dry solids, with less frequent samples if consistently absent over six months.

Reduction in Pathogens During Sludge Treatment
Research Studies

The aforementioned concerns surrounding the safety of recycling sewage sludge to agricultural land sparked a major program of research in the UK. During 1998, UK Water Industry Research Limited (UKWIR), the Environment Agency (EA) and the DETR jointly commissioned research to characterize the risks associated with the beneficial utilization of sewage sludge in agriculture. Administration of the research work is performed by UKWIR. The objective of the research work is to assure the safety of current recycling of treated sewage sludge and application techniques. Specifically:

1. To develop analytical procedures for determining human and animal pathogens in sewage sludge (Verotoxigenic *E. coli*, *salmonellae*, *campylobacter*, *Listeria monocytogenes*, *Cryptosporidium*, *Giardia*, Enteroviruses).

2. To study the fate of pathogens during the treatment of sewage sludge.

3. To establish, by means of a risk assessment methodology, whether current sewage sludge recycling operations have an observable risk with respect to human and animal pathogens.

Phase I (method development) has been completed and reported by the Centre for Applied Microbiology and Research (CAMR), Porton Down (UKWIR, 2001). Investigations into the effect of sludge treatment processes on pathogens (Phase II) was undertaken by a consortium led by the University of Leeds School of Civil Engineering (UKWIR, 2002). The microbiological risk assessment was carried out at WRc-NSF by Paul Gale and colleagues (UKWIR, 2003).

The following sludge treatment processes are being investigated:

1. Mesophilic anaerobic digestion (MAD)

2. Alkaline stabilization

3. Composting

4. Pasteurisation (followed by MAD)

5. Thermal drying

With the exception of thermal drying, all processes were studied at laboratory or pilot scale. The process conditions investigated were the minimum recommended in the Code of Practice (DoE, 1989, 1966) (Table 1) with the exception of MAD which was operated at a mean hydraulic retention of 12 days at 35°C $\pm$ 3°C followed by secondary digestion with a minimum retention of 14 days. Benchmarking exercises were performed to compare the experimental data to the performance of full scale operating plants. The research has generated a large amount of data that is still undergoing statistical analysis and interpretation. Table 5 gives a summary showing the inactivation of pathogens achieved by the treatment processes investigated at laboratory scale

Survey of Operational Plants

The inactivation of indigenous *E. coli* in full-scale sludge treatment processes was investigated during a three-month study carried out in the autumn of 1998. This investigation looked at nine different sludge treatment processes at 35 sites in the UK (UKWIR, 1999). All of the processes surveyed reduced the numbers of *E. coli*. So-called "enhanced" treatment processes such as composting, lime addition and thermal drying, were capable of reducing numbers of *E. coli* to the detection limit of the analytical method. For all of these methods, over 90% of results showed bacterial reductions of 6 $\log_{10}$ or greater. Lagooning of sludge was capable of significantly reducing numbers of *E. coli* and, depending on the method of operation, reductions in the order of 5 $\log_{10}$ were observed. MAD, the process carried out at the majority of sites surveyed, reduced numbers of *E. coli* by, on average, between 1.4 and 2.3 $\log_{10}$ depending on the solids content of the product. For sites producing a liquid product, 2–4% dry solids (ds), 78% of all reductions for *E.* coli were in the range 1 to 2 $\log_{10}$. Where digested sludge was subsequently dewatered to produce a cake, 89% of results showed reductions in the range 2 to 4 $\log_{10}$. The one vermiculture site in the survey showed results intermediate between MAD and the "enhanced" treatment processes (Table 6).

Table 5. Summary of Log_{10} Pathogen Reductions Achieved in Laboratory and Pilot Scale Trial Experiments

Test Organism	Sludge Treatment Process									
	MAD		Lime[2]		Pasteurization[3]				Composting[4]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1		Trial 2		40/55	55/40
					55/240	70/30	55/240	70/30		
E. coli	1.22	1.9	4.76	4.35	7.47	7.47	7.8	7.8	5.9	5.9
S. typhimurium	NP	NP	9.7	8.7	6.72	6.72	6.12	6.12	NP	NP
S. dublin	NP	NP	7.58	6.84	NP	NP	NP	NP	5.2	5.2
S. senftenberg	2.87	NP	6.1	7.95	7.97	7.97	7.9	7.8	5.01	5.07
S. enteriditis	NP	NP	NP	NP	8.24	8.24	NP	NP	4.3	4.3
C. jejuni	NP	NP	7.2	7.5	5.31	5.31	7.18	7.18	5.3	5.3
L. monocytogenes	0.92	NP	6.7	NP	8.99	8.99	1.92	1.92	4.89	4.89
Cryptosporidium	4.56	4.56	2	0	1.03	1.03	NP	NP	NP	NP
Poliovirus[1]	6.2	6.4	6.43	NP	8.47	8.15	8.45	8.08	NP	NP

Key

MAD Mesophilic Anaerobic Digestion
55/240 55°C for 240 mins
70/30 70°C for 30 mins
40/55 40°C for 5 d followed by 4 hr at 55°C
55/40 55°C for 4 hr followed by 40°C for 5 d
NP Not Performed

Notes

(1) Limit of detection for poliovirus 10^3 due to background virus
(2) Lime achieved 100% removal except for poliovirus and Cryptosporidium
(3) Same values for 55/240 and 70/30 indicate 100% removal
(4) 100% removal for all bacteria except S. sentftenberg

Table 6. Reduction in E. coli at Operational Sludge Treatment Facilities in the UK

Treatment	Number of Samples	Log_{10} Reduction in E.coli			Log_{10} E. coli in Treated Sludge (100 g^{-1} dry wt.)		
		25%ile	Mean	95%ile	25%ile	Mean	95%ile
Lagooning	36	1.47	2.65	6.00	5.08	5.93	8.32
MAD, liquid	208	1.04	1.39	2.36	7.10	7.41	8.27
MAD, cake	93	1.76	2.29	3.64	6.27	6.65	7.46
Vermiculture	14	4.2	5.12	6.54	3.20	4.50	5.07
Composting	31	5.75	6.71	9.10	1.05	2.43	4.70
Lime addition	32	5.70	7.10	9.05	0	1.45	3.00
Thermal drying	70	6.52	7.14	8.90	0.33	1.67	3.56

Thermophilic Processes

United Utilities Water has installed an Alpha Biotherm thermophilic aerobic digestion (TAD) system to an existing MAD sludge treatment plant at Ellesmere Port (Cheshire, UK). Details of the installation and its operation have been described (Davies and Messerli, 2000). Briefly, following thickening (to between 4.5-8.6% ds) sludge is heated to approximately 35°C. Sludge is transferred to the TAD reactor where it undergoes mixing and aeration until the target temperature of between 65°C and 70°C is attained. The reactor is locked out for an hour to allow pasteurization to take place. After one hour the treated sludge is cooled via a heat exchanger (to recover heat) and passed forward to the conventional MAD stage. Pasteurization temperatures are achieved through a combination of externally applied heat by means of hot water and biological heat from the exothermic growth of thermophiles in the TAD reactor. Microbiological data from a continuous seven-day commissioning period are shown in Table 7.

Table 7. Performance of TAD Plant at Ellesmere Port (UK)

Median Count (n=7)	Raw Sludge	Ex TAD Plant
Enterobacteriacea (cfu/10g)	$1.9x10^7$	None detected (<100)
E. coli (cfu/10g)	$4.2x10^6$	None detected (<100)
Salmonellae (MPN /100g)	$3.5x10^2$	None detected (<1)

Future Developments

The European Commission has started the process of revising the current directive (86/278/EEC) and has published three working documents. The latest working document was issued on 27 April 2000. It proposes two categories of treatment – Advanced (hygienization) and Conventional. The document specifies the processes in each category (http://europa.eu.int/comm/environment/waste/sludge_en.pdf). Advanced treatments require validation using a test strain of *Salmonella* which exhibits a degree of thermal resistance (*S. senftenberg* W775). The acceptance criterion is a minimum of a 6 log_{10} reduction. In operation the treatment must achieve a 6 log_{10} reduction in *E. coli* to less than 5×10^2 g^{-1} ds. In addition the treated sludge shall not contain *Salmonella* spp. in 50 g (wet weight). Conventional treatment is required to achieve a least a 2 log_{10} reduction in *E. coli*. Details of the sludge treatment processes specified by the Commission are shown in Appendix 1.

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APPENDIX 1: Sludge treatment processes

Extract from 3rd Working Document on Sludge 27 April 2000 (ENV.E.3.1/LM)

Advanced treatments (hygienisation)

- Thermal drying ensuring that the temperature of the sludge particles is higher than 80°C with a reduction of water content to less than 10% and maintaining a water activity above 0.90 in the first hour of treatment;
- Thermophilic aerobic stabilisation at a temperature of at least 55°C for 20 hours as a batch, without admixture or withdrawal during the treatment;
- Thermophilic anaerobic digestion at a temperature of at least 53°C for 20 hours as a batch, without admixture or withdrawal during the treatment;
- Thermal treatment of liquid sludge for a minimum of 30 minutes at 70°C followed by mesophilic anaerobic digestion at a temperature of 35°C with a mean retention period of 12 days;
- Conditioning with lime reaching a pH of 12 or more and maintaining a temperature of at least 55°C for 2 hours;
- Conditioning with lime reaching and maintaining a pH of 12 or more for three months.

The process shall be initially validated through a 6 Log_{10} reduction of a test organism such as *Salmonella Senftenberg W 775*.

The treated sludge shall not contain *Salmonella spp* in 50 g (wet weight) and the treatment shall achieve at least a 6 Log_{10} reduction in *Escherichia coli* to less than 5 x 10^2 CFU/g.

Conventional treatments

- Thermophilic aerobic stabilisation at a temperature of at least 55°C with a mean retention period of 20 days;
- Thermophilic anaerobic digestion at a temperature of at least 53°C with a mean retention period of 20 days;
- Conditioning with lime ensuring a homogenous mixture of lime and sludge. The mixture shall reach a pH of more than 12 directly after liming and keep a pH of at least 12 for 24 hours;
- Mesophilic anaerobic digestion at a temperature of 35°C with a mean retention period of 15 days;
- Extended aeration at ambient temperature as a batch, without admixture or withdrawal during the treatment period(*);
- Simultaneous aerobic stabilisation at ambient temperature(*);
- Storage in liquid form at ambient temperature as a batch, without admixture or withdrawal during the storage period(*). The sludge treatment shall at least achieve a 2 Log_{10} reduction in *Escherichia coli*.

The relevant process parameters shall be monitored at least daily, and preferably continuously if practicable. Records shall be kept and made available upon request to the competent authority for inspection purposes.

European standards for the monitoring of these treatment processes shall be developed. If CEN standards are not available and until they are developed, ISO, international or national standards shall apply.

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*The minimum time length of the treatment shall be laid down by the competent authority taking into consideration the prevailing climatic conditions in the area where the treatment plant is located.

When the competent authority of the concerned Member State is satisfied that a treatment process not listed in this Annex is capable of achieving the same results as the listed treatments, it shall inform the Commission thereof. The Commission, after evaluation of the information provided, may seize the Committee of representatives of Member States. If the opinion of the Committee is positive, the treatment process shall be included in this Annex.

[Without prejudice to other relevant Community legislation, in particular Directive 90/667/EEC on animal waste.]

Workshop Participants

Workshop on Emerging Infectious Disease Agents and Issues Associated with Animal Manures, Biosolids and Other Organic By-Products
Vernon-Manor Hotel, Cincinnati, Ohio; June 4-6, 2001

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Glossary of Terms

Definitions of words used in this report are listed here; the underlined words are defined elsewhere in this glossary.

Aerobic: Living or active in the presence of oxygen. Used in this report to refer especially to microorganisms and/or decomposition of organic matter.

Anaerobic: Living or active in the absence of oxygen, e.g., anaerobic microorganisms.

Animal (And Poultry) Manure: Animal excreta, including bedding, feed and other by-products of animal feeding and housing operations.

Ascaris: Pale colored nematode genus with a round body that is tapered at both ends; parasite of mammals; lives in the intestines of mammals; produces enzyme inhibitors that protect it from host's digestive enzymes. *Ascaris ova (eggs)* have layered shells. The outer tanned, bumpy layer is the mammillated layer and is useful in identification. The mammillated layer is sometimes absent. Eggs that do not possess the mammillated layer are referred to as decorticated eggs. A potentially infective *Ascaris* egg contains a third stage larvae encased in the sheath of the first larval stage.

Bacteria: Single-celled microscopic organisms lacking chlorophyll. Some cause disease, and some do not. Some are involved in performing a variety of beneficial biological treatment processes including biological oxidation, solids digestion, nitrification, and denitrification.

Biodegradable Volatile Solids: The biodegradable portion of total solids that volatilizes to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % g g^{-1}.

Biological Oxidation: The aerobic degradation of organic substances by microorganisms, ultimately resulting in the production of carbon dioxide, water, microbial cells, and intermediate by-products.

Biosolids: The organic solids product of municipal wastewater treatment that has been appropriately treated to control pathogens and vector attractiveness for beneficial utilization; referred to in this publication as treated sewage sludge. Wastewater treatment solids that have received PSRP or PFRP treatment, or their equivalents, according to the Part 503 rule to achieve a Class A or Class B pathogen status. The solids:liquid content of the product can vary, for example: Liquid biosolids 1-4% solids; Thickened liquid biosolids 4-12% solids; Dewatered biosolids (equals cake) 12-30% solids; Dried biosolids >50% solids (advanced alkaline stabilized, compost, thermally dried). In general liquid biosolids and thickened liquids can be handled with a pump. Dewatered/dried biosolids are handled with a pitchfork, shovel or front end loader.

BOD (Biochemical Oxygen Demand): The quantity of oxygen used in the biological and chemical oxidation (decomposition) of organic matter in a specified time, at a specified temperature (typically 5 days at 20°C), and under specified conditions. A standardized BOD test is used in assessing the amount of readily biodegradable organic matter in wastewater.

Buffer: Around the perimeter of a storage or application area, a strip of land that is not intended to receive biosolids or manure. The purpose of the buffer is to provide a protective zone around field boundaries, roads and sensitive areas, such as streams and wet soil areas.

By-Product: A secondary or additional product; something produced in the course of manufacturing the principal product.

Cake: Dewatered biosolids with a solids concentration high enough (>12%) to permit handling as a solid material. (Note: Some dewatering agents might still cause slumping even with solids contents higher than 12%.)

CFU (Colony Forming Units): A term used to enumerate microbes in a sample and based on the fact that the visible cluster (colony) of microbes that appears on nutrient agar medium in a petri dish can develop from a single or group of microbial cells.

Coliform: A lactose-fermenting member of the family *Enterobacteriaceae* commonly associated with the intestinal tract of animals, including humans, fish, birds and insects. However, many are also known and reported to be free-living in the environment and associated with plants and soil. While most coliforms are not medically significant, all are opportunistic pathogens, i.e., cause disease in immunologically compromised individuals. *adj*—Of or relating to the bacilli that commonly inhabit the intestines of human beings and other vertebrates, especially the colon bacillus, *Escherichia coli*.

Composting: The accelerated (controlled and optimized) decomposition of organic matter by microorganisms, which is accompanied by temperature increases above ambient. For biosolids, composting is typically a managed aerobic process.

Denitrification: The conversion of nitrogen compounds to nitrogen gas or nitrous oxide by microorganisms in the absence of oxygen.

Dewatered Biosolids: The solid residue (12% total solids by weight or greater) remaining after removal of water from a liquid biosolids by draining or filtering. Dewatering is distinguished from thickening in that dewatered biosolids may be transported by solids handling procedures.

Digestion: Controlled decomposition of organic matter by microorganisms. Consequent volume and mass reduction in wastewater solids (sewage sludge). Anaerobic digestion produces methane and carbon dioxide, whereas aerobic digestion produces carbon dioxide and water.

Enterococcus: Gram-positive bacteria formerly classified as Group 'D' streptococci. In 1984, several members of the Group 'D' streptococci were reclassified as a new genus, *Enterococcus*, for clinical reasons. *Enterococcus* now represents a small portion of organisms that constitute the fecal streptococcus group. *Enterococcus* may be used as "indicator organisms" for fecal contamination in the same way that Group 'D' streptococci were. *Enterococcus*, or 'enteric cocci', are commonly found in fecal material of humans and a variety of animals. These organisms can survive harsh conditions for longer periods of time in the environment than either total or fecal coliforms, *E. coli* or salmonellae. For example, *Enterococcus* can grow in the presence of 6.5% sodium chloride and at 45°C, and also survive at temperatures as high as 60°C.

Environmental Management System (EMS): A management system in which the principle of constant improvement is applied to environmental endpoints; best-known example is the ISO14001. EMS program developed for biosolids, available through the National Biosolids Partnership (www.biosolids.org), includes 17 system requirements for effectively managing biosolids activities at all critical control points throughout the biosolids "value chain" (i.e., from pretreatment through ultimate disposition of the biosolids product). Four key steps are repeated to drive constant improvement — Plan, Implement, Check, Review. The EMS for biosolids also includes elements for public participation and outreach, which help ensure good management practices are followed.

EQ Biosolids: Exceptional Quality biosolids, meets one of the six Class A pathogen reduction alternatives, and one of the eight Vector Attraction Reduction options, and Part 503, Table 3 high quality pollutant concentration standards.

Fecal Coliform: The type of coliform bacteria present in virtually all fecal material produced by mammals. Since the fecal coliforms may not be pathogens, they only indicate the potential presence of human disease organisms. See indicator organisms.

Fecal Streptococcus: A member of a group of gram-positive bacteria known as *Enterococcus,* previously classified as a subgroup of *Streptococcus.* See "Enterococcus" above.

Field Storage: Temporary or seasonal storage area, usually located at the application site, which holds biosolids destined for use on designated fields. State regulations may or may not make distinctions between staging, stockpiling, or field storage. In addition, the time limits for the same material to be stored continuously on site before it must be land applied range from 24 hours to two years.

Filter Press: Equipment used near the end of the solids production process at a wastewater treatment facility to remove liquid from biosolids and produce a semi-solid cake.

Fungi: Any of numerous eukaryotic organisms of the kingdom Fungi, which lack chlorophyll and vascular tissue and range in form from a single cell to a mass of branched filamentous hyphae (mycelia) that produce specialized fruiting bodies. The kingdom includes the yeasts, smuts, rusts, and mushrooms.

Generator: Person or organization producing or preparing biosolids by treatment of wastewater solids (sewage sludge). Also, a person who, or organization that, changes the sewage sludge's characteristics either through treatment, mixing or any other process.

Good Management Practices: Schedules of activities, operation and maintenance procedures (including practices to control site runoff, spillage, leaks, or drainage), oversight, and other management practices found to be highly effective and practicable in the preparation and safe use of biosolids and in preventing or reducing discharge of pollutants to waters.

Helminth and Helminth Ova: Parasitic worms, e.g., roundworms, tapeworms, *Ascaris, Necator, Taenia*, and *Trichuris*, and ova (eggs) of these worms. Helminth ova are quite resistant to disinfection, and can be passed out in the feces of infected humans and organisms and ingested with food or water. One helminth ovum is capable of hatching and growing when ingested.

Heterotroph: An organism that is dependent on complex organic substances for synthesizing cellular components.

Hydraulic Loading Rates: Amount of liquid or sewage sludge applied to a given treatment process and expressed as volume per unit time, or volume per unit time per surface area.

Indicator Organisms: Microorganisms, such as fecal coliforms and fecal streptococci (*Enterococcus*), used as surrogates for bacterial pathogens when testing sewage sludge, manure, compost, leachate and water samples. Indicator microbes are generally not pathogenic, but co-exist in habitats with pathogens. Tests for the presence of the surrogates are used because they are relatively easy, rapid, and inexpensive compared to those required for pathogens, such as *Salmonella* bacteria.

Infiltration: The rate at which liquid enters the soil surface, expressed in inches per hour, influenced by both permeability and moisture content of the soil.

Lagoon: A reservoir or pond built to contain liquid/slurry, sediment and/or manure usually containing 4% to 12% solids until they can be removed for application to land.

Land Application: The spreading or spraying of biosolids onto the surface of land, the direct injection of biosolids below the soil surface, or the incorporation into the surface layer of soil; also applies to manure and other organic residuals.

Leachate: Liquid which has come into contact with or percolated through materials being stockpiled or stored; contains dissolved or suspended particles and nutrients.

Liquid Biosolids or Manure: Biosolids or animal manure containing sufficient water (ordinarily more than 88 percent) to permit flow by gravity or pumping.

Mercaptans: A group of volatile chemical compounds that are one of the breakdown products of sulfur-containing proteins; noted for their disagreeable odor.

Microorganism: Bacteria, fungi (molds, yeasts), protozoans, helminths, and viruses. The terms *microbe* and *microbial* are also used to refer to microorganisms, some of which cause disease, and others that are beneficial. Parasite and parasitic refer to infectious protozoans and helminths. Microorganisms are ubiquitous, possess extremely high growth rates, and have the ability to degrade all naturally occurring organic compounds, including those in water and wastewater. They use organic matter for food.

Mineralization: The process by which elements combined in organic form in living or dead organisms are eventually reconverted into inorganic forms to be made available for a new cycle of growth. The mineralization of organic compounds occurs through oxidation and metabolism by living microorganisms.

Mitigation: The act or state of reducing the severity, intensity, or harshness of something; to alleviate; to diminish; to lessen; as, to mitigate heat, cold, or odor.

MPN (Most Probable Number): A statistically derived approximation of the number of microorganisms per unit volume or mass of sample. Often used to report the number of coliforms per 100 ml wastewater or water, but applicable to other microbial groups as well. The power of the MPN estimate increases with the number of analyses performed per sample.

Municipal Wastewater: Household and commercial water discharged into municipal sewer pipes; contains mainly human excreta and used water; distinguished from solely industrial wastewater.

Natural Attenuation: Destruction of pathogens in the ambient environment, usually over an extended time period, during which exposure to extremes of temperature, competition/interactions with soil organisms — including predators, UV irradiation, desiccation, toxicity, and starvation — ensue. Negative biological factors directly impacting survival of the target pathogens are competition, predation, hyperparasitism, and antibiosis.

Nitrification: The biochemical oxidation of ammonia nitrogen to nitrate nitrogen, which is readily used by plants and microorganisms as a nutrient.

Nonpoint Source: Any source, other than a point source, discharging pollutants into air or water.

Nonpoint Source Pollution: Any alteration of the chemical, physical, biological, or radiological integrity of water or air, originating from any source other than a point source.

Nutrient: Any substance that is assimilated by organisms and promotes growth; generally applied to nitrogen and phosphorus in wastewater, but also other essential trace elements or organic compounds that microorganisms, plants, or animals use for their growth.

Nutrient Management Plan: A series of good management practices aimed at reducing agricultural nonpoint source pollution by balancing nutrient inputs with crop nutrient requirements. A plan includes soil testing, analysis of organic nutrient sources such as biosolids, compost, or animal manure, utilization of organic sources based on their nutrient content, estimation of realistic yield goals, nutrient recommendations based on soil test levels and yield goals, and optimal timing and method of nutrient applications.

Odor Character: The sensory quality of an odorant, defined by one or more descriptors, such as fecal (like manure), sweet, fishy, hay, woody resinous, musty, earthy. See *Atlas of Odor Character Profiles*, 1985.

Odor Dilutions To Threshold or D/T: Dimensionless unit expressing the strength of an odor. An odor requiring 500 binary (2-fold) dilutions to reach the detection threshold has a D/T of 500. An odor with a D/T of 500 would be stronger than an odor with a D/T of 20.

Odor Intensity: A measure of the perceived strength of an odor. This is determined by comparing the odorous sample with "standard" odors comprised of various concentrations of n-butanol in odor-free air.

Odor Pervasiveness: Persistence of an odor; how noticeable an odorant is as its concentration changes; determined by serially diluting the odor and measuring intensity at each dilution.

Odor Threshold: Detection - The minimum concentration of an odorant that, on average can be detected in odor-free air. Recognition - The minimum concentration of an odorant that, on average, a person can distinguish by its definite character in a diluted sample.

Off-Site Storage: Storage of biosolids at locations away from the wastewater treatment plant or from the point of generation. Several terms encompass various types of storage: Stockpiling, Field Storage, and Storage facility.

Organic Matter: In soil, compost, and residuals, the partially or nearly completely decayed plant, animal, and microbial biomass (exclusive of living macro-fauna and macro-flora); also, in general, substances that contain organic carbon.

Overland Flow: Refers to the free movement of liquid/slurry over the ground surface, e.g., runoff.

Pathogen: Disease-causing organism, including certain bacteria, fungi, helminths, protozoans, or viruses.
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Permeability: The rate of liquid movement through a unit cross section of saturated soil in unit time; commonly expressed in inches per hour.

PFRP, PSRP: See Process to Further Reduce Pathogens, or Process to Significantly Reduce Pathogens.

pH: A measure used to indicate the degree of acidity or alkalinity of a substance. The pH is expressed as the $\log_{10}$ of the reciprocal of the actual hydrogen ion concentration. The pH ranges from 0-14, where 0 is the most acidic, 14 is the most alkaline, and 7 is neutral.

Phytotoxin: Any substance having a toxic or poisonous effect on plant growth. Immature or anaerobic compost can contain volatile fatty acids that are phytotoxic to plants. Soluble salts can also be phytotoxic, in addition to toxic heavy metals and toxic organic compounds.

Point Source: Any discernable, confined, or discrete conveyance from which pollutants are or may be discharged, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, stack, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft.

Polymer: A compound composed of repeating subunits used to aid in flocculating suspended particulates in wastewater into large clusters. This flocculation aids solids removal and enhances the removal of water from slurries during dewatering.

Process To Further Reduce Pathogens (PFRP): The process management protocol prescribed by the U.S. EPA used to achieve Class A biosolids in which pathogens are reduced to undetectable levels. Composting, advanced alkaline stabilization, chemical fixation, drying or heat treatment, are some of the processes that can be used to meet Part 503 requirements for Class A.

Process To Significantly Reduce Pathogens (PSRP): The process management protocol prescribed by the U.S. EPA used to achieve Class B biosolids in which pathogen numbers are significantly reduced, but are still present. Aerobic and anaerobic digestion, air drying and lime stabilization are typical PSRP processes.

Protozoa: Single-celled, microorganisms; many species can infect humans and cause disease. The infective forms are passed as cysts or oocysts in the feces of humans and animals and accumulate in flocculated solids; they are quite resistant to disinfection processes, such as chlorination, that eliminate most bacteria, but are susceptible to destruction by drying. Examples are *Cryptosporidium parvum* and *Giardia lamblia*.

Retention Time: The period of time wastewater or sewage sludge takes to pass through a particular part of a treatment process, calculated by dividing the volume of processing unit by the volume of material flowing per unit time.

Risk, Potential: Refers to a description of the pathways and considerations involved in the occurrence of an event (or series of events) that may result in an adverse health or environmental effect.

Risk Assessment: Is a quantitative measure of the probable occurrence of an adverse health or environmental effect. Involves a multi-step process that includes hazard identification, exposure assessment, dose-response evaluation, and risk characterization. The latter combines this information so that risk is calculated. Risk = Hazard x Exposure

Salmonella: Rod-shaped bacteria of the genus *Salmonella*, many of which are pathogenic, causing food poisoning, typhoid, and paratyphoid fever in human beings, or causing other infectious diseases in warm-blooded animals, and can cause allergic reactions in susceptible humans, and sickness, including severe diarrhea with discharge of blood.

Septage: Domestic sewage (liquid and solids) removed from septic tanks, cesspools, portable toilets, and marine sanitation devices; not commercial or industrial wastewater.

Sewage, Domestic: Residual liquids and solids from households conveyed in municipal wastewater sewers; distinguished from wastewater carried in dedicated industrial sewers. See Wastewater.

Solids: In water and wastewater treatment, any dissolved, suspended, or volatile substance contained in or removed from water or wastewater.

Stability: The characteristics of a material that contribute to its resistance to decomposition by microbes, and to generation of odorous metabolites. The relevant characteristics include the quantity of biodegradable matter present, nutrient, moisture and salts content, pH, and temperature. For biosolids, compost, or animal manure, stability is a general term used to describe the quality of the material taking in to account its origin, processing, and intended use.

Stockpiling: Holding of biosolids at an active field site long enough to accumulate sufficient material to complete the field application.

Storage: Placement of Class A or B biosolids in designated locations (other than the WWTP) until material is land applied; referred to as field storage. See also Off-Site Storage.

Storage Facility: An area of land or constructed facilities committed to hold biosolids until the material may be land applied at on- or off-site locations; may be used to store biosolids for up to two years. However, most are managed so that biosolids come and go on a shorter cycle based on weather conditions, crop rotations and land availability, equipment availability, or to accumulate sufficient material for efficient spreading operations.

Threshold Odor: See Odor Threshold

Treated Sewage Sludge: Refers to sewage sludge that has gone through a pathogen and vector attraction reduction treatment process. It essentially is the same material as biosolids, however the US EPA has not officially adopted the term "biosolids" in the Part 503 rule, therefore official EPA documents continue to use the term sewage sludge.

Turbulence: Irregular atmospheric motion especially characterized by up and down currents. Increasing turbulence results in dilution of odors.

VAR: Abbreviation for Vector Attraction Reduction

Vector: An agent such as an insect, bird or animal, that is capable of transporting pathogens.

Virus: A microscopic, non-filterable biological unit, technically not living, but capable of reproduction inside cells of other living organisms, including bacteria, protozoa, plants, and animals.

Volatile Compound: A substance that vaporizes at ambient temperature. Above average heat can increase the volatilization (vaporization) rate and amount of many volatile substances.

WWTP: Abbreviation for wastewater treatment plant.

Wastewater Treatment: The processes commonly used to render water safe for discharge into a waterway: 1) Preliminary treatment includes removal of screenings, grit, grease, and floating solids; 2) Primary treatment includes removal of readily settlable organic solids; 50-60% suspended solids are typically removed along with 25-40% BOD; 3) Secondary treatment involves use of biological processes along with settling; 85-90% of BOD and suspended solids are removed during secondary treatment; 3) Tertiary treatment involves the use of additional biological, physical, or chemical processes to remove remaining nutrients and suspended solids.

Water Holding Capacity: Percentage of water-filled pore volume relative to the total volume of water saturated material, percent pore water (v)/total water (v).