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United States
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Problem Formulation for Human Health Risk Assessments of Pathogens in Land-applied Biosolids

National Center for Environmental Assessment
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NOTICE

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ABSTRACT

This document provides concepts and planning considerations for conducting human health risk assessments on potential pathogens in land-applied biosolids. As one component of U.S. EPA's Action Plan for setting new priorities for it's biosolids program, this document summarizes the existing literature; defines critical pathogen stressors; develops conceptual models linking the most likely stressors, pathways and health responses of concern; evaluates the overall quality and utility of available risk assessment data; highlights existing tools and methodologies; and provides an outline of an Analysis Plan that identifies gaps in knowledge and research and methods needed to provide more scientifically defensible assessments relevant to U.S. EPA's decision needs.

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LIST OF ABBREVIATIONS

CFR	Code of Federal Regulations
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
HPC	Heterotrophic plate counts
ICC-PCR	Integrated cell-culture PCR
ILSI	International Life Sciences Institute
MAD	Mesophilic anaerobic digestion
NRC	National Research Council
PCR	Polymerase chain reaction
PM_{10}	Particulate matter with a diameter between 2.5 and 10 micrometers
PSRP	Process to significantly reduce pathogens
R_0	Reproductive number (R naught)
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
SDWA	Safe Drinking Water Act
U.S. EPA	United States Environmental Protection Agency
UV	Ultraviolet

PREFACE

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA) prepared this document for Federal, State, and Local risk managers and assessors, contractors, and other interested parties who may be interested in conducting microbial risk assessments on land-applied biosolids.

In 2002, the National Research Council (NRC) released a report entitled: "*Biosolids Applied to Land: Advancing Standards and Practices"* which was a review of EPA's regulation "The *Standards for the Use or Disposal of Sewage Sludge"* (otherwise known as the "Part 503 Rule" found in Title 40 of the Code of Federal Regulations published in 1993). In 2003, EPA released a final action plan for setting new priorities for the biosolids program which included the Agency's response to the NRC report called "*Standards for the Use or Disposal of Sewage Sludge; Final Agency Response to the National Research Council Report on Biosolids Applied to Land and the Results of EPA's Review of Existing Sewage Sludge Regulations"* (68 FR 75531).

This present report follows-up on one project listed in EPA's action plan; specifically, to provide a problem formulation and analysis plan relating to uncertainties associated with conducting quantitative microbial risk assessments on land-applied biosolids (68 FR 75540). In particular, this work focuses on critical human health assessment endpoints and potential pathogens that should be considered; develops conceptual models linking the most likely stressors, pathways and health responses of concern; evaluates the overall quality and utility of available risk assessment data, tools and methodologies; and develops an analysis plan suggesting additional research and methods for improving risk assessments in this topical area and supporting EPA's decision needs.

This report is based on the results of a literature review and summary first conducted in 2008 and then updated again in 2010 based on feedback received from an expert panel of independent, external peer reviewers and from public comments. The intent of this document is to improve the planning of future risk assessments of land-applied biosolids and to assist in the design of specific research to fill current data gaps on this important environmental topic.

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EXECUTIVE SUMMARY

Approximately 3.4 million tons of biosolids, dry weight, are land-applied annually to farms, forests, rangelands, mine lands, and other land use types (Pepper et al., 2006; NRC, 2002). Biosolids are defined by the U.S. Environmental Protection Agency (EPA) (U.S. EPA, 1995) as "the primarily organic solid product yielded by municipal wastewater treatment processes that can be beneficially recycled" as soil amendments. Concerns for potential human health effects from land-applied biosolids can be addressed through the conduct of a risk assessment. This report focuses on the systematic planning step (a "problem formulation" defining the major factors to be considered) for risk assessments of pathogens in land-applied biosolids. This report follows the common problem formulation steps of hazard identification, conceptual model development, and the development of an analysis plan. A review of pathogens in biosolids literature forms the basis of this report. The intended use of this document is to assist in the development of future risk assessments and to identify specific research needed to fill current data gaps.

Policy Background

In 1993, EPA's *Standards for the Use or Disposal of Sewage Sludge* (Title 40 of the Code of Federal Regulations Part 503) standardized the land application practices of biosolids. Risk-based limits were established only for chemicals. Regulations for pathogens in biosolids were developed based on existing methods for detection. Since the promulgation of the Part 503 rule, a body of scientific data and methodologies has become available for re-examination of EPA's management of microbial pathogens in land-applied biosolids. In 2002, the National Research Council (NRC) convened a review of the Part 503 rule by the Committee on Toxicants and Pathogens in Biosolids applied to land and published a report entitled, *Biosolids Applied to Land: Advancing Standards and Practices*. The report identified a critical need to update the scientific basis of the Part 503 rule and made recommendations for EPA to improve the biosolids program. This problem formulation document represents one of many responses to the NRC report since that time (U.S. EPA, 2003b).

Scope

This document focuses on potential microbial risks to human health by pathogens in land-applied biosolids. The particular product of concern is Class B biosolids originating from human waste, not Class A biosolids which are treated to reduce the numbers of pathogenic organisms to below limits of detection. Considerations of animal waste are excluded from the scope of this problem formulation. Pathogens that may be present in Class B biosolids include bacteria, viruses, protozoa, and helminthes (see Section 2, Hazard Identification). Exposure routes of inhalation, ingestion, and dermal exposure leading to human infection and diseases are discussed in Section 3, but issues relating to occupational risks do not fit under the regulatory authority of EPA and, as such, are not included in this report. The conceptual models illustrated and narrated here are meant to be generic and broadly useful, and can be adapted for use in site-specific risk assessments. Overall, five scenarios of common public concern are described:

1) Neighboring residences and schools
2) Farm Residents
3) Pica child
4) Drinking water consumers of groundwater
5) Drinking water consumers of surface water

The following assumptions are made in the conceptual models developed for this generic problem formulation document:

- Class B biosolids have higher potential levels of human pathogens than Class A biosolids
- All sewage treatment practices meet standards
- All sewage treatment technologies operate as intended
- Storage facilities are bounded by effective physical barriers

- Modifications to the conceptual models presented here; for example, to relax or add to the assumptions above, or to account for other concerns (e.g., secondary transmission, etc.), can be considered on a case-by-case basis by users of this document

This document provides useful suggestions, but does not serve as guidance for how one would conduct any specific risk assessment. The analysis plan chapter (see Section 5) describes exposure measures, detection methods, data quality needs, dose-response models, and uncertainty analysis. Research needs for future defensible risk assessments are identified without prioritization in the Appendix.

Utility

Major products of this problem formulation document are the generic conceptual models illustrating key relationships between potential pathogens in land-applied biosolids and human health. Risk assessors may use these to develop conceptual models more applicable to their particular scenarios. Risk managers may also use this document to help develop the meaningful questions that risk assessors need to address in order to reach a health protective outcome. The analysis plan will benefit researchers as a summary of the measures, methods, and data needs for improving risk assessment. Researchers will also likely find the Appendix useful as an organized summary of current, peer-reviewed scientific studies relevant to land-applied biosolids.

1. INTRODUCTION

In January 2004, the U.S. Environmental Protection Agency (U.S. EPA) released a final action plan for setting new priorities for the biosolids program, which included the Agency's response to the National Research Council (NRC) report entitled *Biosolids Applied to Land: Advancing Standards and Practices* (NRC, 2002). This report serves as an important step in the Agency's response to the NRC report. Presented here is a development of a problem formulation and analysis plan for conducting quantitative microbial risk assessments on land-applied biosolids. For risk assessment the NRC recommends seeking stakeholder input. For this document stakeholder input was gathered through public comments and peer review.

This report summarizes the existing literature on the microbial risks to humans posed by pathogens in land-applied biosolids (see the Appendix); defines critical microbial hazards; develops conceptual models linking the most likely hazards, pathways and health responses of concern; evaluates the overall quality and utility of available risk assessment data, tools and methodologies; and develops an analysis plan which identifies the research and methods required for providing a scientifically defensible risk assessment relevant for EPA's decision needs.

"Problem formulation is a systematic planning step that identifies the major factors to be considered in a particular assessment" (U.S. EPA, 2003a). It was developed for ecological risk assessment and subsequently adopted for cumulative human health risk assessments (U.S. EPA, 1998, 2003a). The principal products of problem formulation are a conceptual model and an analysis plan (U.S. EPA, 2003a).

As a guide to problem formulation development, this report organizes current literature and presents conceptual models for a generic framework. For use in specific cases, further refining of information is recommended. Two particular audiences may find this document most helpful. First, assessors who seek to assess risks to human health from land-applied biosolids can use this generic problem formulation as a basis for developing their own problem formulations. It can point users to information sources, serve an introduction to the relevant literature, and facilitate the design process for different types of risk assessments. Second, researchers and research planners can

use the research needs identified in this report to select and prioritize research projects related to pathogens in biosolids. It can also help researchers to understand how to design their studies so as to generate results relevant to risk assessment.

There are many examples of microbial risk assessments conducted by Federal agencies. The risk assessment performed for the *Long Term 2 Enhanced Surface Water Treatment Rule*, for example, has all the major features of a quantitative microbial risk assessment using the human health chemical risk assessment framework also suggested here (U.S. EPA, 2006b).

Figure 1 illustrates a generalized framework for human health risk assessments (adapted from ILSI, 2000). Note that the document you're reading now focuses on the Problem Formulation (Model Development) stage shown in Figure 1. Some of the major sources of information on risk assessment frameworks evaluated during the development of this problem formulation document include the following:

- National Academy of Sciences, National Research Council, *Risk Assessment in the Federal Government: Managing the Process* (NRC, 1983);
- National Academy of Sciences, National Research Council, *Science and Decisions: Advancing Risk Assessment* (NRC, 2009);
- EPA Office of Water/International Life Sciences Institute (ILSI) Risk Science Institute *Revised Framework for Microbial Risk Assessment* (ILSI, 2000);
- EPA *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998); and
- EPA Lessons Learned on Planning and Scoping for Environmental Risk Assessments (U.S. EPA, 2002a).

Problem formulation was developed as part of the ecological risk assessment framework. However, it has been adapted to human health risk assessment in various contexts including cumulative risk assessment (U.S. EPA, 2003a) and microbial risk assessment (ILSI, 2000). The National Academy of Sciences has recommended that human health risk assessors emulate ecological risk assessors in problem formulation (NRC, 2009).

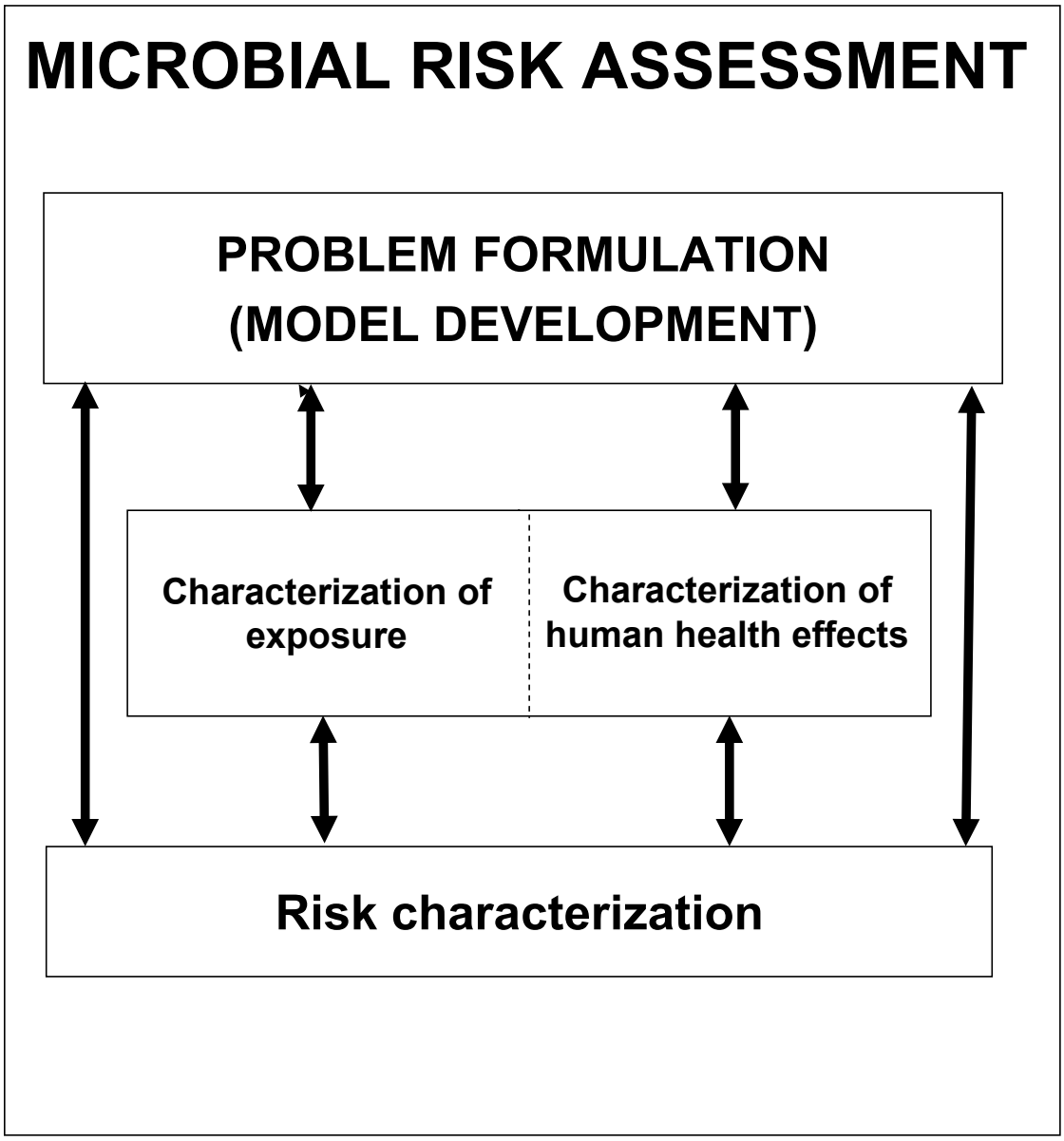

FIGURE 1

General Framework for Assessing the Risks of Human Diseases Following Exposure to Pathogens

Source: Adapted from ILSI (2000).

1.1. SCOPE

This report focuses on land applied Class B biosolids, which are defined by a combination of treatment requirements and subsequent site restrictions. Class A biosolids are not specifically considered in this document because they are treated to reduce the numbers of pathogenic organisms to below limits of detection. Furthermore, Class A biosolids generally have a reduced level of biodegradable components that could attract vectors and are thus generally unregulated for use in commerce. It is assumed here that, based on applicable treatment regimes, Class B biosolids have a greater potential for higher levels of microbial pathogen hazards than Class A biosolids. Occupational exposure is not addressed in this document because the Occupational Safety and Health Administration is the primary agency responsible for regulating human health risks in occupational settings. Studies of untreated animal manures are also beyond the scope of this report. This problem formulation document also focuses on microbial hazard risk assessment planning and therefore does not include discussions of chemical or physical hazards potentially associated with land applied biosolids.

Although EPA's Guidelines for Ecological Risk Assessment (U.S. EPA, 1998) were considered during the development of this document, the scope of this document is limited to human health and does not include any ecological effects of land applied biosolids. Furthermore, scenarios that include noncompliance with existing regulations are not included.

2. HAZARD IDENTIFICATION

Hazards[1] are agents that may adversely affect human health or other assessment endpoints. The identification of hazards is a necessary precursor to developing conceptual models, especially for risk assessments of a complex substance like biosolids. Similar to what EPA (1998) describes to be important questions to answer in ecological risk assessments, a human health risk assessment for microbial pathogens in biosolids should consider the following:

1. What is the source of the pathogens?
2. What is the spatial extent of the source?
3. What types of hazards are present: bacterial, viral, or others?
4. What are the modes of action of the hazards?

Essentially, hazards and their sources should be identified and characterized well enough to inform the development of conceptual models, as well as, the more detailed exposure pathways covering all reasonable exposure scenarios relevant to a particular case. For example, pathogens in bioaerosols come from a different source than those that remain in biosolids-amended soil particles. The resultant differences in pathogen fate and transport and other factors can all be discussed in problem formulation documentation.

This report focuses on pathogens and endotoxins originating in biosolids. For use in the development of a specific risk assessment, assessors should include, in addition to descriptions of microorganisms in biosolids, aspects of the biosolids matrix that affect pathogenicity, and dimensions of the source that affect how exposure is modeled or monitored.

This chapter describes the source of biosolids, including the components of the mixture, the extent of the source, the matrix, the Class B treatment process, site

[1] In human health risk assessment the term “hazard” has been adopted and in ecological risk assessment the term “stressor” is used.

restrictions, and vector attraction reduction options. Following the description of the source, this chapter provides pertinent information about bacterial, viral, protozoan, and helminth pathogens, as well as endotoxins that may be present in biosolids and may cause adverse effects to human health.

2.1. SOURCE

Approximately 3.4 million tons of biosolids, dry weight, are land-applied annually to farms, forests, rangelands, mine lands and other land use types. Only about 0.1% of available agricultural land in the United States is treated with biosolids (Pepper et al., 2006; NRC, 2002). These soil amendments are thought to have beneficial nutrients for plant growth as well as components to improve the physical properties of soils. The EPA did not use the term biosolids in the Part 503 rule, but EPA (1995) defines biosolids as “the primarily organic solid product yielded by municipal wastewater treatment processes that can be beneficially recycled” as soil amendments. The NRC’s definition of biosolids is “sewage sludge treated to meet the land-application standards in the Part 503 rule or any other equivalent land application standards” (NRC, 2002). Pathogen standards are technologically based requirements “aimed at reducing the presence of pathogens and potential exposures to them by treatment or a combination of treatment and use restrictions” (NRC, 2002).

Biosolids are complex mixtures that contain organic and inorganic compounds and organisms from wastewaters of households, commercial and industrial facilities, as well as compounds added or formed during wastewater treatment processes (NRC, 2002). Inorganic and organic contaminants in biosolids are also described in NRC (2002) and may include metals, trace elements, polychlorinated biphenyls, dioxins, pharmaceuticals, surfactants and other contaminants. Class B biosolids are host to a microbial community that potentially includes human pathogens, whereas Class A biosolids have been treated to reduce the numbers of human pathogens to below detectable levels, Thus, risks to human health from potential pathogens in Class B biosolids are the primary focus of this problem formulation.

2.1.1. Spatial Extent of Source

Risk assessors need to characterize the areal extent of biosolids application or storage that is specific to a particular risk assessment. Biosolids may be localized (e.g., a pile of stored biosolids is a continuous point source), more diffuse (e.g., a land applied field is a continuous source that may be classified as a point source or a nonpoint source), or mobile (e.g., a biosolids applicator is a short term source that moves from location to location). Pathogen transport models may be specific to the spatial extent of the source and include considerations for proximity of pathogens to humans. Large piles of biosolids that serve as temporary storage before placement can represent continuous, localized sources of pathogen-containing bioaerosols (described below) (Dowd et al., 2000). Similarly, bioaerosols can be created during the transport of biosolids from one location to another at a site, during the 'front-end loading' or "shoveling" of biosolids from one pile to another, or from the lifting of biosolids-amended soil particles by strong winds (Pillai, 2007). Areas of application may be large fields or more localized windrows. If the risk assessment is intended to estimate cumulative risk, then biosolids application in adjacent fields (to the site where maximum exposure occurs) over time may be pertinent. A risk assessment may even address larger spatial scales, for example, the entire area treated with biosolids either nationally, or by state.

2.1.2. Reproduction

In addition to providing physical reservoirs of pathogens, biosolids and biosolids-amended soils can serve as sources of additional pathogens if some of the bacterial organisms reproduce (Zaleski et al., 2005a). Evidence about regrowth or lack of regrowth of particular species in Class B biosolids is important information to include in a conceptual model.

2.1.3. Matrix

Four principal biosolids-containing matrices are possible sources of pathogens: liquid biosolids, solid biosolids, biosolids-amended soil and bioaerosols created from biosolids. Bioaerosols emerged as an important potential source of pathogens during

the literature review for this report and are therefore important to consider during the problem formulation stage of risk assessments pertaining to land-applied biosolids.

1. *Liquid biosolids.* Liquid biosolids are the texture of muddy water and usually contain 2−8% solids (Paez-Rubio et al., 2007). They are expensive to transport.

2. *Solid biosolids*. Biosolids cake (15–30% solids content) (Paez-Rubio et al., 2007; Meckes, 2011) is dewatered biosolids with the texture of a wet sponge (Virginia Department of Health, 1999).

3. *Biosolids-amended soil.* Over repeated applications, biosolids-amended soil has different physical properties from soil alone. The altered physical properties of soil include increased water holding capacity, water infiltration and stability of soil aggregates (Brown and Henry, 2002).

4. *Bioaerosols.* Bioaerosols are aerosolized biological particles that vary from 0.02−100 μm in diameter. They are formed when dewatered biosolids are loaded into application equipment or when liquid and dewatered biosolids are spread onto land (Paez-Rubio et al., 2007). The following information comes from references in Pillai and Ricke (2002) and Pillai (2007). The size, composition and concentration of microbial populations comprising aerosols vary with biosolids source, method of application and meteorology and other environmental conditions at the biosolids application site. Bioaerosols generated from water sources (e.g., liquid biosolids) usually have a thin layer of moisture surrounding clusters of microorganisms. Bioaerosol particles have a net charge that depends on the source characteristics and can affect deposition rates. Factors that control bioaerosol transport include the size, density and shape of particles or droplets, as well as wind speed, relative humidity and temperature. When some aerosolized bacteria are exposed to high relative humidity, they sorb water, which protects the cells from inactivation by ultraviolet (UV) light (Peccia et al., 2001).

2.1.4. Treatment

A description of the sewage sludge treatment process provides risk assessors with information about the potential pathogen content of biosolids. Treatment methods are intended to reduce the volume and organic content of biosolids and to reduce the number of pathogens while retaining beneficial properties for fertilization and other soil amendment and land reclamation purposes (NRC, 2002). The Part 503 rule defines two categories of biosolids: Class A biosolids, which have no detectable concentrations of pathogens, and Class B biosolids, which have detectable concentrations of

pathogens (U.S. EPA, 1993). The example conceptual models presented in this report focus on Class B biosolids, which are defined by a combination of treatment requirements and site restrictions. The treatment of these biosolids must meet one of three criteria: fecal coliform count of less than 2×10^6/gram of dry solids at the time of disposal, treatment by a process to significantly reduce pathogens (PSRP), or treatment by a process equivalent to a PSRP. In the absence of standardized methodologies for developing risk-based processes, five operational-based processes in the Part 503 Rule (listed below) were determined to be PSRPs, based on their resulting fecal coliform concentrations less than 2×10^6/gram of dry solids and their ability to reduce *Salmonella* and enteric virus levels by a factor of 10 (U.S. EPA, 1999):

1. Aerobic digestion at specific combinations of time and temperature
2. Air drying for three months, with average ambient daily temperatures above freezing for at least two months
3. Anaerobic digestion for specific combinations of time and temperature
4. Composting for specific combinations of time and temperature
5. Lime stabilization to give a pH greater than 12 after 2 hours of contact

Fecal coliforms are enteric bacteria that are used as indicators of the potential for the presence of bacterial pathogens. *Salmonella* species are human pathogens. Changes in indicator and pathogen loads before and after the promulgation of the Part 503 rule have been documented (Pepper et al., 2008b). In this problem formulation, it is assumed that treatment requirements and site restrictions meet standards. If sewage sludge is dewatered, thickening agents such as ferric chloride, lime, or polymers are added (NRC, 2002).

2.1.5. Site Restrictions

Site restrictions also provide information about the content of biosolids to which humans are exposed, because, although pathogens attenuate over time, soil amended with Class B biosolids may not be considered free from pathogens for at least one year

following treatment (Gibbs et al., 1997). Site restrictions are required to reduce contact with Class B biosolids until environmental exposures such as heat and desiccation have decreased concentrations of bacterial, viral and helminth pathogens to below detectable concentrations equivalent to those in Class A biosolids (NRC, 2002). Natural attenuation also incorporates biological factors such as competition, predation, hyperparasitism (growth of a secondary microorganism in or on the primary pathogen or parasite) and antibiosis (Smith et al., 2005a). Site restrictions to public access, grazing and harvesting are included (see Table 1).

2.1.6. Vector Attraction Reduction

The Part 503 rule requires that one of twelve management options be used to control disease vectors. These are described in detail in the rule and in NRC (2002): volatile solids reduction, specific oxygen uptake rate, anaerobic bench-scale test, aerobic bench-scale test, aerobic process for compost, pH adjustment, drying without primary solids, drying with primary solids, injection, and incorporation. The first eight options are process-based options, the first five of which are intended to contribute to long-term stabilization through the degradation of putrescible organics. Injection of biosolids and incorporation within six hours of application are considered physical barriers to vector attraction.

2.2. PATHOGENS

A variety of bacterial, viral, protozoan, and helminth pathogens may be present in Class B biosolids. The relative quantity of pathogen shedding in the contributing human population will influence the prevalence of pathogens in biosolids. Therefore, fecal borne pathogens that are rare in the United States might be less of a concern than endemic pathogens and a pathogen that is responsible for a community outbreak situation. In a study conducted over 20 years (1986–2006), Zerzghi et al. (2009) reported no bacterial or viral pathogens were detected in soil samples collected from biosolid amended plots in December (10 months after the last land application) demonstrating that pathogens introduced via Class B biosolids survived in soil only transiently.

TABLE 1

Site Restrictions for Class B Biosolids (40 CFR 503)*

40 CFR 503.14 Management Practices
(a) Bulk sewage sludge shall not be applied to the land if it is likely to adversely affect a threatened or endangered species listed under Section 4 of the Endangered Species Act or its designated critical habitat.
(b) Bulk sewage sludge shall not be applied to agricultural land, forest, a public contact site, or a reclamation site that is flooded, frozen, or snow-covered so that the bulk sewage sludge enters a wetland or other waters of the United States, as defined in 40 CFR 122.2, except as provided in a permit issued pursuant to section 402 or 404 of the CWA.
(c) Bulk sewage sludge shall not be applied to agricultural land, forest, or a reclamation site that is 10 meters or less from waters of the United States, as defined in 40 CFR 122.2, unless otherwise specified by the permitting authority.
(d) Bulk sewage sludge shall be applied to agricultural land, forest, a public contact site, or a reclamation site at a whole sludge application rate that is equal to or less than the agronomic rate for the bulk sewage sludge, unless, in the case of a reclamation site, otherwise specified by the permitting authority.
40 CFR 503.32[b][5] Pathogens
(i) Food crops with harvested parts that touch the biosolids/soil mixture and are totally above the land surface shall not be harvested for 14 months after application of biosolids.
(ii) Food crops with harvested parts below the surface of the land shall not be harvested for 20 months after application of biosolids when the biosolids remain on the land surface for four months or longer prior to incorporation into the soil.
(iii) Food crops with harvested parts below the surface of the land shall not be harvested for 38 months after application of biosolids when the biosolids remain on the land surface for less than four months prior to incorporation into the soil.
(iv) Food crops, feed crops and fiber crops shall not be harvested for 30 days after application of biosolids.
(v) Animals shall not be grazed on the land for 30 days after application of biosolids.
(vi) Turf grown on land where biosolids is applied shall not be harvested for one year after application of the biosolids when the harvested turf is placed on either land with a high potential for public exposure or a lawn, unless otherwise specified by the permitting authority.
(vii) Public access to land with a high potential for public exposure shall be restricted for one year after application of biosolids.
(viii) Public access to land with a low potential for public exposure shall be restricted for 30 days after application of biosolids.

*http://yosemite.epa.gov/r10/water.nsf/NPDES%20Permits/Sewage%20S825/$FILE/503-032007.pdf.
CFR = Code of Federal Regulations; CWA = Clean Water Act.

Risk assessors should consider and list the range of possible pathogens in the problem formulation, though it may be necessary to focus on only a limited number based on the specifics of each individual risk assessment. For example, the source, transport and fate of pathogens in applied biosolids may be considered, including: (1) the origin of the human waste, (2) the likelihood of each pathogen to survive sewage treatment, and (3) their fate in soil. For additional considerations about these and other pathogen factors, see the EPA Office of Water's *Draft Protocol for Microbial Risk Assessment to Support Human Health Protection for Water-Based Media* (U.S. EPA, 2009a). Many of the potential pathogens in Class B biosolids and the diseases they may cause are summarized in Table 2. Due to the location and objective of each study cited, researchers who list principal pathogens of concern in sewage sludge and/or biosolids do not always list the same organisms (Dudley et al., 1980; NRC, 2002; Gerba and Smith, 2005; Pepper et al., 2006, 2010; Epstein, 2006; Yanko, 2005). Unlike chemical hazards, pathogens can multiply and some bacteria can reproduce outside of the host organism under favorable environmental conditions. The types and levels of pathogens in biosolids are determined by the incidence of infection within the biosolids generating community and the type of treatment process used (Straub et al., 1993). The biosolids matrix (i.e., biosolids, biosolids-amended soil, bioaerosols, or biosolids particles in water) may affect the fate of pathogens, and therefore determine exposure.

Additional factors to consider for conducting risk assessments may include the accessibility, attenuation, and the bioavailability of the pathogens, as well as site factors such as underlying geology, soil type, and depth to groundwater. Considerations for screening pathogens from inclusion are presented for each class of pathogens throughout the remainder of this section. It should be noted that, in general, pathogens without dose-response data are not candidates for quantitative microbial risk assessment. However, qualitative microbial risk assessment would still be possible. References that provide helpful information for prioritizing pathogens of concern include Sidhu and Toze (2009) and Smith et al. (2005a).

For risk assessors who are familiar with chemical risk assessment it is important to note that pathogens differ from chemicals in several ways that are important for risk assessment. Some of these differences include:

- Microbial Growth and Death—Some pathogens can multiply in the environment and all multiply in hosts. Different species, and even different strains within a species, grow and die in unique patterns. In contrast, although chemicals can bioaccumulate and bioconcentrate, they are not known to multiply in the environment or hosts. Both chemicals and pathogens can decrease due to environmental factors; chemicals can be transformed or degrade and pathogens can die.

- Detection Methodologies—Generally, methods for detecting chemical pollutants are sufficiently sensitive to detect and quantify concentrations well below the levels that are known to have human health effects. This is not necessarily the case for pathogens. Theoretically, a single pathogenic organism can cause infection (and lead to illness). Analytical methods for detecting low levels of pathogens (e.g., one organism) are not sufficiently developed to be reliable.

- Genetic Diversity of Pathogens—Microorganisms are genetically diverse and allelic ratios in a population can change significantly within a few generations. In addition, microbial genomes can evolve quickly (within days or weeks) through mutation or horizontal gene transfer. Pathogens in biosolids would presumably be reflective of the pathogen status of the generating community.

- Host Immunity and Susceptibility—Infection and illness due to pathogens is, in some cases, highly dependent on the immune status of the individual, which can fluctuate based on time since last exposure, presence of concurrent infections (e.g., human immunodeficiency virus [HIV]), and a number of other factors (e.g., life stages, gender, genetics). Although body weight, age, and metabolic capacity differences are considered in the development of chemical criteria, genetic and acquired differences in susceptibility are not usually considered.

Secondary Transmission—Microbial infections can be transmitted from an individual to other susceptible individuals, and even to some animals. With the exception of the mother-fetus relationship, chemicals in tissues of exposed individuals are not known to transmit to other individuals. Chemicals that are on exposed individuals' clothing or skin can be transferred to household and other contacts. But this type of chemical exposure would not be amplified compared to the primary exposed individual, whereas pathogen secondary transmission can amplify the disease incidence.

TABLE 2

Example Pathogens of Potential Concern in Sewage Sludge and Biosolids

Class	Organism	Disease or Symptoms
Bacteria	*Listeria monocytogenes*	Meningitis, encephalitis, septicemia, intrauterine or cervical infections with abortion
	Helicobacter pylori	Stomach ulcers, gastritis, increased risk of stomach cancer
	Campylobacter jejuni	Gastroenteritis
	Pathogenic *Escherichia coli*	Gastroenteritis, hemolytic uremic syndrome
	Shigella spp.	Bacillary dysentery
	Salmonella spp.	Salmonellosis (food poisoning), typhoid/paratyphoid fever
	Yersinia spp	Yersiniosis (gastroenteritis)
	Legionella spp.	Severe respiratory illness, mild flulike illness
Viruses	Astroviruses	Gastroenteritis
	Rotaviruses	Gastroenteritis
	Caliciviruses	Gastroenteritis
	Adenoviruses	Respiratory diseases, gastroenteritis
	Hepatitis virus A-E	Infectious hepatitis, liver inflammation, hepatic cancer
Helminth Parasites	*Taenia spp.*	Nervousness, enteric distress, abdominal pain, anorexia, insomnia
	Ascaris lumbricoides	Digestive disturbances, abdominal pain, transitory liver and lung disease
	Trichuris spp.	Gastrointestinal distress, anemia
	Toxocara canis	Fever, abdominal discomfort, neurological symptoms

Table 2 (cont.)		
Class	Organism	Disease or Symptoms
Protozoan Parasites	*Cryptosporidium parvum*	Diarrhea
	Giardia lamblia	Fever, diarrhea
	Cyclospora	Diarrhea, nausea, vomiting and abdominal cramps
	Microsporidia	Diarrhea
	Entamoeba histolytica	Dysentary, colitis
	Balantidium coli	Diarrhea, constipation, abdominal pain

Sources: Dudley et al. (1980), Gerba and Smith (2005), Epstein (2006), NRC (2002), Pepper et al. (2006) and Bowman and Fayer (2005).

2.2.1. Bacteria

2.2.1.1. *Salmonella*

All serotypes of this genus are pathogenic to humans and cause symptoms ranging from mild gastroenteritis to severe disease and death. In the United States, salmonellosis is mainly due to foodborne transmission because the bacteria found in beef and poultry are able to grow in foods (Pepper et al., 2006). *Salmonella* can apparently survive during sewage treatment and grow in biosolids under some conditions (Sahlstrom et al., 2006). Class A biosolids allows for growth of Salmonella under anaerobic concentrations (Zaleski et al., 2005a). Because of this potential for growth, Pepper et al. (2006) argue that *Salmonella* are the bacteria of greatest concern in Class B biosolids, although Skanavis and Yanko (1994) concluded a low probability of infection in most scenarios. In 40 Code of Federal Regulations (CFR) 503, *Salmonella* are the bacterial pathogen indicators for biosolids quality.

2.2.1.2. *Escherichia coli O157:H7*

Escherichia coli is found in the intestinal tract of humans and most warm-blooded animals, and most strains are not pathogenic. However, several strains can cause gastroenteritis. The greatest concern in the United States is enterohemorrhagic *E. coli* of the serotype O157:H7 (Pepper et al., 2006). The organism has been spread in contaminated drinking water, through recreational water exposure, and contaminated food (Yanko, 2005; Pepper et al., 2006; Barker et al., 1999). Cattle manure is the most significant source of exposure, but the organism has been detected in biosolids too (Lytle et al., 1999; Pepper et al., 2006).

2.2.1.3. *Campylobacter jejuni*

This pathogen is the principal cause of bacterial diarrheal illness in the United States (Mead et al., 1999). Food is the major source of infection. Little research has been conducted to investigate the occurrence of *Campylobacter* in sewage sludges, biosolids, or the environment (Yanko, 2005), though a few studies of raw and treated sludge are reviewed in Pepper et al. (2006).

2.2.1.4. *Shigella Spp.*

Bacteria of this genus are closely related to *E. coli.* The bacteria are frequently found in water contaminated with human sewage and are transmitted by the fecal-oral route. Salads, raw vegetables, milk and dairy products and poultry sometimes are contaminated with *Shigella* (Pepper et al., 2006). *Shigella* is more infective than most enteric bacteria and secondary attack rates are high among children (Haas et al., 1999). In addition to transmission in day care facilities, *Shigella* has also been transmitted through unchlorinated wading pools, water fountains, food items such as parsley and bean dip, men who have sex with men (CDC, 2008a), and surface waters due to bather shedding (WHO, 2004). However, *Shigella spp.* do not survive well in the environment or after treatment of biosolids. Therefore, they are not likely to be a significant problem (Pepper et al., 2006).

2.2.1.5. *Yersinia Spp.*

These bacteria cause gastroenteritis with diarrhea or vomiting, fever and abdominal pain. *Yersinia enterocolitica* has been detected in environmental sources such as ponds and lakes, though the major source of infection in the United States is pork products (Pepper et al., 2006). Waterborne outbreaks have also occurred. In Japan, infections of *Y. pseudotuberculosis* from contaminated water and foods have been reported. The bacterium has been detected in raw, digested and dewatered biosolids (Straub et al., 1993), but little information is available about background levels or survival in soils or waters (Pepper et al., 2006).

2.2.1.6. *Listeria monocytogenes*

This bacterium causes foodborne diseases, primarily in immunocompromised people such as pregnant women. It can cause encephalitis, meningitis and intrauterine or cervical infections (Epstein, 2006). *L. monocytogenes* has been detected in activated and anaerobically digested biosolids (Watkins and Sleath, 1981; DeLuca et al., 1998). The bacterium is widespread in the environment (Yanko, 2005).

2.2.1.7. *Helicobacter pylori*

This bacterium is the principal cause of stomach ulcers and is associated with increased risk of stomach cancer.[2] *H. pylori* may be the most common cause of bacterial infection in humans (up to 90% of some populations are infected, Epstein 2005), though rates of infection are decreasing (Yanko, 2005). The source of many infections is vegetables irrigated with untreated wastewater (Brown, 2000). The digestive tract of humans is apparently the main reservoir of *H. pylori* (Yanko, 2005). Whether *H. pylori* is present in Class B biosolids is unknown (Pepper et al., 2006).

2.2.1.8. *Legionella*

Infections with *Legionella* can result in a life-threatening respiratory illness, Legionnaires' Disease, especially in immunocompromised people or the elderly, or a mild illness called Pontiac Fever. Outbreaks of *Legionella* usually occur through airborne transmission of bacteria from hot water in building cooling towers or other aerosolizing devices (Yanko, 2005). High concentrations have been measured in biosolids at a food industry sewage treatment plant where workers contracted Pontiac Fever (Gregersen et al., 1999; Yanko, 2005). Moreover, Yanko (2005) speculates that the bacteria should grow well in "warm, self-composting organic masses." However, there is no known case of either Legionnaires' Disease or Pontiac Fever associated with the production or land application of biosolids.

2.2.1.9. *Screening Bacterial Pathogens*

Some bacteria may be excluded from consideration in risk assessments of pathogens in biosolids. For example, some experts believe that *Staphylococcus aureus* "are not a likely source of…human exposure or infection" (Pepper et al., 2006). In a study of 23 biosolids samples (16 Class B samples) from 15 U.S. sites, none contained *S. aureus* (Rusin et al., 2003a). Similarly, analyses of 37 air samples were also negative for the bacterium (Rusin et al., 2003a). However these studies have been criticized because of the technical difficulties associated with organic matter-associated

[2] National Cancer Institute, available online at: http://www.cancer.gov/cancertopics/factsheet/risk/h-pylori-cancer (accessed 6/30/10).

pathogen extraction (Lewis and Gattie, 2003). Although there is little information on the fate of *Vibrio cholera* in biosolids treatment or land application, Yanko (2005) recommends that the low incidence of this disease in the United States (0−5 cases per year) is a good justification for focusing research on other pathogens.

2.2.1.10. *Ranking Bacterial Pathogens*

Risk assessors may also prioritize bacterial pathogens of concern in risk assessments of land-applied biosolids. A workgroup of biosolids experts developed methods for evaluating 20 potential pathogens in biosolids (see Chapter 4 in Smith et al. [2005a]). They considered several factors such as a pathogen's public health significance (number of infections or severity of disease), prevalence in biosolids and sewage sludge, survival during wastewater treatment and the availability of appropriate analytical methods. Similar criteria might be used by risk assessors in the problem formulation.

2.2.2. Viruses

Over 140 types of enteric viruses are excreted by humans and are likely to be present in municipal wastewater. Viruses are almost always detected in Class B biosolids and by definition are not detected in Class A biosolids (Pepper et al., 2008a; Gerba et al., 2002). Examples covered here include enteroviruses, rotaviruses, caliciviruses, adenoviruses, astroviruses, and picornaviruses that may cause Hepatitis A and E.

2.2.2.1. *Enteroviruses*

Enteroviruses include polioviruses, coxsackieviruses, and echoviruses. Both fecal-oral and respiratory routes of infection are common. Enteroviruses are commonly isolated from untreated biosolids. Generally, they are reduced by 90% or more during the aerobic and anaerobic digestion of sludge which produces Class B biosolids (Pepper et al., 2006).

2.2.2.2. *Rotaviruses*

These are the only double-stranded ribonucleic acid (RNA) viruses transmitted through water to humans (NRC, 2002). Along with caliciviruses, rotaviruses are the leading cause of gastroenteritis in the United States (Monroe et al., 2000; CDC, 2008b) and a major cause of hospitalization of children in the United States (Gerba et al., 1996a). These viruses cause waterborne and foodborne outbreaks in the United States. They have been detected in wastewater, but little information is available regarding their occurrence in biosolids (NRC, 2002).

2.2.2.3. *Caliciviruses*

Caliciviruses may be the leading cause of water and foodborne illness in the world and are a leading cause of viral gastroenteritis (Monroe et al., 2000). The two genera are the *Norovirus* (previously known as Norwalk viruses) and *Sapovirus* (an example species being Sapporo virus) (NRC, 2002).[3] Recently researchers have demonstrated that a highly differentiated 3-D cell culture model can support the natural growth of human noroviruses, which is a major breakthrough in tools that will enable the future study of noroviruses (Straub, 2007).

2.2.2.4. *Adenoviruses*

These common and persistent viruses in wastewater (NRC, 2002) are the second most common cause of childhood viral diarrhea (Gerba et al., 1996a). NRC (2002) provides references indicating that recreational and drinking waters are pathways of exposure for adenoviruses. Adenoviruses are present in untreated sewage sludge (Gerba et al., 2002). Enteric adenoviruses have been detected in Class B biosolids (NRC, 2002; Pepper et al., 2008a), and adenovirus type 40 has been detected in anaerobically digested biosolids (NRC, 2002). Along with hepatitis A virus, adenovirus is the most thermally resistant virus (Gerba et al., 2002). Using polymerase chain reaction (PCR) human adenovirus genomes were found in 88% of the Class B

[3] Virus taxonomy: available online at: http://talk.ictvonline.org/files/ictv_documents/m/msl/1231.aspx (accessed 6/30/10).

biosolids sampled (Viau and Peccia, 2009). Overall, little is known about the removal of adenoviruses by Class B treatment processes (Gerba et al., 2002).

2.2.2.5. *Astroviruses*

These viruses are a cause of gastroenteritis, primarily in children. Foodborne and waterborne outbreaks have occurred in the past. They have been found in biosolids (Chapron et al., 2000), though little is known about their removal by Class B treatment processes (Gerba et al., 2002).

2.2.2.6. *Hepatitis A*

This picornavirus is responsible for infectious hepatitis. This pathogen is transmitted by food and water, and primarily infects the liver. The highest infection rate is among children 5–14 years old (CDC, 1999). Along with adenoviruses, Hepatitis A is the most thermally resistant virus (Gerba et al., 2002). No information is available on the prevalence of Hepatitis A in biosolids.

2.2.2.7. *Hepatitis E*

This picornavirus, transmitted by the fecal-oral route, has been responsible for major waterborne disease outbreaks in developing countries but has also been reported frequently in travelers to those regions. It is the major cause of acute viral hepatitis in developing countries (Gerba, 2005). Symptoms include jaundice, fatigue, abdominal pain and nausea. Hepatitis E is a more serious infection than Hepatitis A, with case fatalities of 2–3% in the general population and 20–30% in pregnant women (Haas et al., 1999). No information is available on the prevalence of Hepatitis E in biosolids.

2.2.2.8. *Screening Viral Pathogens from Consideration*

Some viruses may be excluded from consideration in biosolids by pathogen risk assessors. For example, a workgroup on viruses in biosolids concluded that blood-borne viruses such as HIV would be likely to be inactivated during wastewater or biosolids treatment (Smith et al., 2005b). This workgroup also concluded that lipid-containing viruses have low viability in water and may not survive wastewater or

biosolids treatment. However, they recommended that lipid-containing viruses such as rhinoviruses, influenza viruses and herpes viruses not be excluded from consideration until it is known whether any survive treatment (Smith et al., 2005b).

2.2.3. Protozoa

Cryptosporidium and *Giardia* are the predominant diarrhea-causing protozoan parasites transmitted through food and water in the United States. These parasites of the small intestine have infective, environmentally resistant stages called cysts (for *Giardia*) or oocysts (for *Cryptosporidium*). Pepper et al. (2006) review studies in which *Cryptosporidium* and *Giardia* have been detected in sewage sludge and biosolids. Oocysts do not survive under low moisture or high heat conditions, and therefore would be expected to be inactivated during treatment and land application (Whitmore and Robertson, 1995). This expectation has been confirmed by Bowman et al. (2000), who found that these protozoa died within days of Class B biosolids treatment. However Pepper et al. (2006) suggest that new cell culture methods are needed to assess protozoan oocyst viability and confirm that these organisms do not present a hazard in biosolids.

Additional protozoa could be present in sewage sludge and/or biosolids (Bowman and Fayer, 2005). *Cyclospora* causes diarrhea, nausea, vomiting and abdominal cramps. *Toxoplasma gondii* causes neurologic flu-like symptoms, retinitis and if mothers are infected for the first time while pregnant the fetus can suffer death, brain damage, hydrocephaly, and numerous less severe symptoms (APHA, 2004). Some genera of Microsporidia cause diarrhea. *Entamoeba histolytica* causes severe dysentery and extra-intestinal abscesses. *Balantidium coli* causes diarrhea and constipation, but Bowman and Fayer (2005) suggest that their presence is less likely in biosolids than that of other protozoa. Life histories of all of these species, as well as potential effects of biosolids treatment, are summarized in Bowman and Fayer (2005).

Bowman and Fayer (2005) consider the potential hazards of various protozoa by summarizing information on settling rates in wastewater and considering potential resistance to disinfection. “Soft-shelled” protozoa (*Balantidium*, *Entamoeba* and *Giardia*) will probably persist in effluents but not in biosolids. The Apicomplexan

protozoa (*Cryptosporidium*, *Cyclospora*, *Toxoplasma*) probably react similarly (but sometimes uncertainly) to the effects of different disinfection methods but settle at different rates. Microsporidia have not been studied much in the context of biosolids treatment (Bowman and Fayer, 2005).

2.2.4. Helminths

Several helminth species potentially occur in biosolids. Eggs of many helminth species probably settle in wastewater, are resistant to sewage treatment methods, and end up in biosolids (Bowman and Fayer, 2005).

2.2.4.1. *Trichuris trichiura*

Trichuris (whipworm) is a genus of nematode that is parasitic in the cecum and large intestine of mammals. It causes diarrhea. Human infections result from ingestion of infected eggs. Eggs in wastewater would be expected to settle rapidly and be found in sewage sludge wherever infected people are present in the community (Bowman and Fayer, 2005). Eggs are not likely to be damaged by the quantities of ultraviolet, ozone, or chlorine used for disinfection in wastewater treatment processes.

2.2.4.2. *Ascaris lumbricoides*

Ascaris is a genus of nematode that is parasitic in the small intestine. Adult worms may develop within the small intestine and cause digestive disturbances. Transitory liver and lung disease is caused by larval migration (Bowman and Fayer, 2005). Human infections with *Ascaris lumbricoides* result from ingestion of infectious *Ascaris* eggs usually from soil or produce grown in soil containing *Ascaris* eggs (APHA, 2004). Although *Ascaris* eggs appear to be present at very low densities in biosolids and perhaps even in raw sewage sludge (NRC, 2002), the eggs of *Ascaris* are an indicator in biosolids because of their resistance to most treatment processes and representativeness of helminth egg viability.

2.2.4.3. *Taeniid Tapeworm Eggs*

The life histories of taeniid tapeworms require a carnivore final host in which the small intestine is infected (Bowman and Fayer, 2005). For *Taenia solium* the final host

is humans or pigs. For *Taenia saginata*, eggs passed in the stool of humans are only infectious to cattle. Human infection is from the ingestion of raw or undercooked beef containing the larval stage (APHA, 2004). The adult worms cause few or no symptoms in humans, but eggs can develop to a larval stage (cysticercus) that can cause central nervous system and enteric symptoms. Although *Taenia* species are usually acquired from ingestion of infected beef or pork, the eggs of this pathogen have been detected in some biosolids (Barbier et al., 1990).

2.2.5. Endotoxins

Endotoxins are nonspecific lipopolysaccharide-protein complexes created from the cell walls of gram-negative bacteria (DeLuzio and Friedman, 1973). They consist of polysaccharide chains connected by a core oligosaccharide to a lipid portion, consisting of a series of long-chain fatty acids, connected by amide and ester linkages to a phosphorylated diglucosamine structure (Epstein, 2006). They may become airborne when dried, pulverized to micrometer and submicrometer size particles, and agitated (Smith et al., 2005a). In the bloodstream these toxins may cause a broad range of physiological effects, including fever, coughing, breathlessness, flu-like symptoms, inflammation, and shock (Yanko, 2005; Pepper et al., 2006; Epstein and Moss, 2006). Endotoxins are relatively heat stable (Epstein, 2006).

Endotoxins have been measured in studies of air at composting plants, though no evidence of residential impact was found because levels decreased to background concentrations beyond site boundaries (Clark et al., 1983; Pepper et al., 2006). Ambient levels of dust-associated endotoxin are high (Smith et al., 2005a; Pepper et al., 2006). Endotoxin levels in Class B biosolids are similar to concentrations in animal manures and composts (Brooks et al., 2006). Farming activities, such as driving a tractor across a field, can result in comparable levels of aerosolized endotoxins as those from land application of biosolids (Brooks et al., 2004a). In fact, most bacteria aerosolized during land application are soil borne in origin (Brooks et al., 2007b). In contrast, low concentrations of endotoxins were present in groundwater at two sites where wastewater was applied to land (Yanko, 2005).

2.2.6. Emerging Pathogens

The lists of pathogens covered in this document should not be considered exhaustive. New pathogens are continually being identified or found in new areas for several reasons such as: changes in the way foods are produced, the global transportation of food and people, advances in molecular biology that permit the identification of new pathogens and their sources, the evolution of pathogens, aging demographics, and the use of microbial risk assessment to quantify risks from environmentally transmitted pathogens (Gerba and Smith, 2005). Emerging pathogens are novel pathogens that have not previously been characterized or established and have only recently been considered hazards of concern in particular media. Gerba et al. (2002) designated *E. coli* O157:H7, *H. pylori* and *L. monocytogenes* as newly emerging bacterial pathogens of potential concern in biosolids. Yanko (2005) points out that many of these emerging bacterial pathogens do not fit the classic fecal-oral transmission pattern. The NRC listed *Mycobacterium*, *E. coli* O157:H7, *Legionella*, *Listeria* and Microsporidia as emerging pathogens likely to be present in biosolids and Adenovirus, Norovirus, Astrovirus, Hepatitis A, Rotavirus and Hepatitis E as emerging viral pathogens likely to be present as well (NRC, 2002). Gerba (2005) listed several emerging viruses without speculating which are likely to be in biosolids, including: picobirnaviruses, picotrinaviruses, coronaviruses, and toroviruses. Yates and Yates (2007) added selected bacteria, viruses and parasites to water and/or microcosms to simulate Class A and Class B treatment. They observed that organisms surviving in the highest numbers or numbers representing the highest risk included *E. coli, Clostridium perfringens* spores, *Listeria innocua* and bacteriophage phi X174.

NRC (2002) identified criteria for selecting emerging pathogens for which additional information on occurrence, persistence, and risk is justified, and for which additional regulations may be needed. These criteria are useful for selecting pathogens on which to focus the hazard characterization in a risk assessment:

- Reliable viability assay
- Wastewater-related disease-causing agents

- Extent of existing data on probability of surviving biosolids treatments (organisms surviving at high pH above 11–12 and heat resistance are of greatest concern)
- Extent of survival in the environment

Based on these criteria, NRC (2002) recommended *E. coli* O157:H7, adenovirus 40, astrovirus, hepatitis A virus and rotavirus in biosolids as priorities for analysis. Caliciviruses would have been selected as a priority if methods for assessing viability were available (NRC, 2002). With the recent publication on a dose-response relationship for noroviruses (Teunis et al., 2008a), caliciviruses are now ready to become priority pathogen for analysis. *Legionella* also merits investigation, but current detection methods are inefficient, difficult to use and expensive (NRC, 2002).

2.2.7. Multiple Hazards

Microbial risk assessors typically assume that microbial pathogens act independently of each other and that the probability of an adverse effect from one type of pathogen is independent of the probability of an adverse effect from another. However, microbial risk assessors may want to consider exposures to pathogens in biosolids at offsite locations or other sources that are not the direct subject of a biosolids risk assessment. This may allow for estimation of the risks of infectious disease from biosolids combined with other sources of the same infectious disease.

There is no evidence to suggest that pathogens and chemicals such as metals in biosolids have interactive effects in humans. However, Lewis et al. (2002) suggested that chemical contaminants in biosolids might irritate the skin and mucous membranes, thereby weakening the first line of defense in the human host, leading to an increase in pathogen host susceptibility. In addition, other constituents in biosolids (e.g., chemicals, metals) may have effects on human immune status (Germolec et al., 1991). Modeling tools have not been developed that include nuances of human immune status due to factors either associated with biosolids or not associated with biosolids. However, the potential for such effects could be discussed when characterization of host susceptibility is presented.

3. DEVELOPMENT OF CONCEPTUAL MODELS, ENDPOINTS AND SCENARIOS

A conceptual model for a risk assessment is a representation of the assumed relationships between sources and effects (Suter, 1999) or between hazards and assessment endpoints (U.S. EPA, 1998). Multiple models may be developed for multiple scenarios. The written descriptions of the risk hypotheses, accompanied by diagrams (termed conceptual models) that illustrate the key relationships, are among the primary products of the problem formulation (U.S. EPA, 1998). Conceptual models "provide a framework for prediction and are the template for generating more risk hypotheses." They form the basis for developing quantitative exposure and effects models for the risk assessment. The models tend to emphasize exposure pathways, including indirect exposures, over mechanisms of effects. Conceptual models have been developed for human health risk assessments of pathogens in biosolids that include detailed source descriptions, transport pathways and routes of exposure (Colford et al., 2003; Eisenberg et al., 2004, 2005, 2006).

For this report, EPA developed conceptual models illustrating the potentially important human exposure pathways for pathogens in biosolids that have been applied to land. These models are developed in response to NRC's assertion that "EPA should develop a conceptual site model to identify the major and minor exposure pathways (including secondary transmission) by which humans might come into contact with pathogens in biosolids" (NRC, 2002). The models are applicable to biosolids amendments to cropland, pasture land, forests, mineland (for reclamation), or other uses. The conceptual models presented here are limited to primary transmission, i.e., exposure of humans to pathogens from biosolids without an intermediate human host. Secondary transmission is infection by pathogens that were shed by infected people. This problem formulation does not provide detailed advice concerning incorporating estimates of secondary infection because the process is not unique to pathogens in biosolids. This does not mean that secondary transmission of pathogens in this context is assumed to be unimportant. For example, pathogens with a high basic reproductive number (R_0), have a high potential for secondary transmission[4] (Heffernan et al., 2005)

[4] A helpful explanation of R_0 can be found at http://wiki.medpedia.com/Influenza_Disease_Transmission.

and might merit consideration of susceptible, infected, and resistant (SIR) populations-type modeling approaches (Smieszek, 2009) for problem formulation. A tiered strategy for a risk assessment could include initial screening for data on R_0, and use of primary disease modeling for pathogens of low transmissibility and consideration of also including secondary transmission modeling for pathogens of high transmissibility. A variety of mathematical models have been formulated, mathematically analyzed, and applied to infectious disease transmission (Hethcote, 2000).

The conceptual models presented in this report are not meant to imply that the risk assessor must assume that adverse health effects are caused by exposure to pathogens in land-applied biosolids. Rather, the models present potential pathways for consideration and supporting evidence on a case-by-case basis. In addition, the conceptual models do not consider contributions from background pathogens not associated with biosolids.

This chapter first presents an overall, general conceptual model for risks from pathogens in land-applied biosolids (see Figure 2), as well as a narrative description of the model. The model is a cascade of processes and states (Suter, 1999) that indicates the mechanisms by which the pathogen hazards potentially contact human hosts to produce infection and disease. A description of the source (methods and rates of land application), environmental fate and transport processes, routes of exposure, host susceptibility factors, infection, and disease are also included. Next, five additional exposure scenarios are presented to demonstrate how Figure 2 (the general conceptual model) can be tailored to more specific scenarios of interest. All the conceptual models presented here may be modified as more knowledge is available on a case-by-case basis.

Each conceptual model contains routes of exposure considered to be potentially significant in many instances. Additional routes may be considered when there are different concerns, or more specific concerns requiring more detail. For example, indirect routes (not included here) involving human consumption of livestock, dairy products, wildlife, fish or shellfish exposed to pathogens could be added. Conversely,

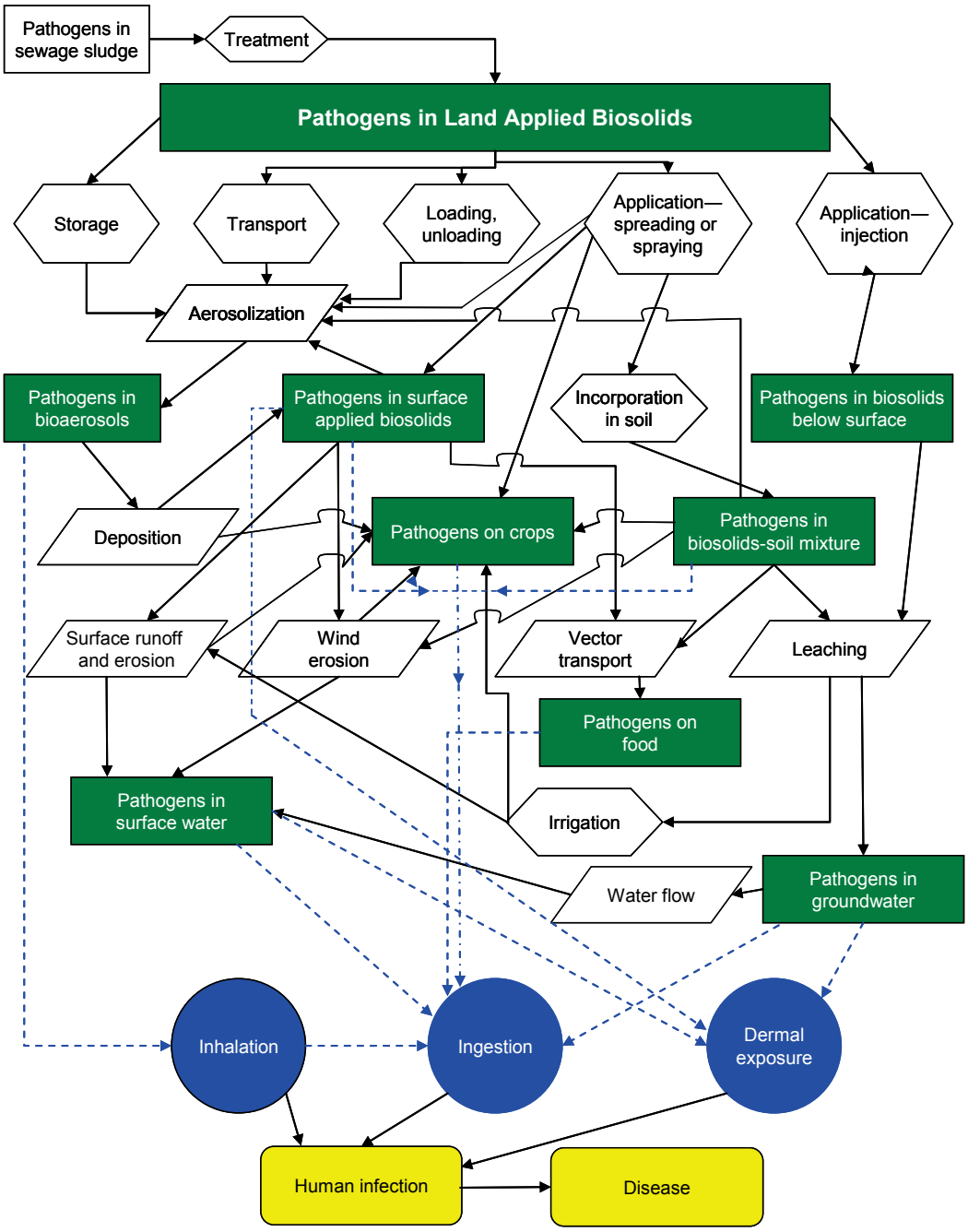

FIGURE 2

General Conceptual Model of the Potential Risks from Pathogens in Land-Applied Biosolids

Legend: Green rectangles: source of pathogens, white hexagons: human actions, white parallelograms: natural process, blue circles: exposure pathways, yellow rectangles with rounded corners: potential responses.

some routes may be deleted if enough information is available to rule them out scientifically.

3.1. PREAPPLICATION PROCESSES

Various treatment processes are not separate boxes in the conceptual model because all treatment technologies are assumed to be operating as intended, generating Class B biosolids (see Figure 2). Human actions in the conceptual model include storage; transport within a site; loading and unloading; land application by spreading, spraying, incorporation or injection in soil; and irrigation (see Figure 2).

Biosolids storage, transport within a site, and loading and unloading processes have been observed to generate bioaerosols (Pillai, 2007; Paez-Rubio et al., 2007; Figure 2). Biosolids are stored when the ground is frozen, during inclement weather, periods of equipment breakdown, or crop growth periods (Evanylo, 1999). Dewatered biosolids are stockpiled, and liquid biosolids may be stored in digesters, tanks, lagoons or drying beds (Evanylo, 1999). Regulations may specify the type of storage facility for long-term storage and require a barrier to prevent the erosion of biosolids or the surface runoff or leaching of pathogens. However, if risk assessors determine that leaks of biosolids or pathogens from storage facilities are feasible, then additional pathways can be included in the conceptual model (e.g., arrows between “Storage” and “Surface runoff and erosion” and/or “Leaching” in Figure 2).

3.2. APPLICATION

3.2.1. Methods of Land Application of Biosolids

The three major methods of biosolids application are injection, surface application without incorporation into soil, and surface application with incorporation into soil. Methods depend on the water content of biosolids, land use, site topography, quantity of debris, presence of obstructions such as trees, presence of waterways, climate, the availability of application equipment (NRC, 2002; Brown and Henry, 2002), and state or local regulations (e.g., Solano County, California requires incorporation of biosolids into soil). The application method is an important determinant of bioaerosol generation, chemical odor, and ultraviolet inactivation of pathogens (NRC, 2002).

Subsurface injection of liquid biosolids involves small-diameter injection tubes to minimize soil disturbance or disking if soil turnover is desired in farm management practices (NRC, 2002). Injection is typically at a depth of 6–9 inches (15−23 cm) and usually occurs before planting or after harvest (NRC, 2002). Injection reduces odor and risk of runoff to surface water (NRC, 2002) as well as prevents aerosolization of biosolids (see Figure 2). As would be expected, Gerba et al. (2002) found that injected biosolids presented a much lower risk of infection from ingestion than surface-applied biosolids without incorporation. Hence, injection is treated separately from surface application in the conceptual model (see Figure 2). Injection can be used on slopes up to a 15% grade (Evanylo, 1999), dependent on state or local laws. This application method serves as a physical barrier that satisfies vector-control requirements (U.S. EPA, 1993). Injection or soil incorporation is rarely used for pasture or hay crops. Application under any circumstance is prohibited for any land use when the ground is frozen (U.S. EPA, 1993).

Surface application involves the application of liquid biosolids or cake solids to the soil surface. Liquid biosolids are typically pumped and sprayed through a cannon or spray nozzle. Solid biosolids are flung from a manure-type spreader or dumped from a truck. Where application is to a forest, a portion of the sprayed biosolids may coat tree surfaces prior to washing down to soil surfaces. In some climates and at high depths of biosolids (thick land applications), drying of the material may require a complete summer period. Drying can be promoted by seeding with a grass such as annual rye or wheat that can germinate and survive in fairly anaerobic conditions (Brown and Henry, 2002). In contrast to injection, surface application is commonly used for hay crops and winter applications. Stabilization of biosolids to meet vector-control requirements must occur through treatment prior to surface application. Surface application may permit ultraviolet inactivation of viruses (NRC, 2002). Spreading of dewatered biosolids may sometimes produce higher bioaerosol emission rates than spraying of liquid biosolids (Paez-Rubio et al., 2007).

Incorporation of cake biosolids into soil through plowing or disking at a depth of 6−9 inches (15−23 cm) may follow surface application (NRC, 2002) and partial drying (Evanylo, 1999). The method is usually used before planting or after harvest (NRC,

2002). Surface application with incorporation is generally limited to soils with less than a 7% slope (Evanylo, 1999), additional state and local laws notwithstanding. Incorporation serves as a physical barrier that satisfies vector-control requirements (U.S. EPA, 1993).

Application methods vary with region and type of biosolids. In the arid and semiarid southwest, liquid anaerobic-digested biosolids are typically injected into the soil subsurface (NRC, 2002). On pasture land, the material tends to be applied to the soil surface, as incorporation is more difficult than on crop land (NRC, 2002). Similarly, incorporation is not common in forests. In many agricultural lands, biosolids cakes are disked into soil (NRC, 2002).

3.2.2. Rates of Land Application of Biosolids

Biosolids are applied at a rate equal to or less than the agronomic rate (the key determinant being the nitrogen requirements of crops, trees, or other vegetation). Rates of application are generally calculated on a dry weight basis. Information on application rates from the 1980s is summarized in Table 3. Notably, the rate of application at reclamation sites is usually much higher than that at farm sites (NRC, 2002). However, agricultural sites are more likely to involve multiple applications (NRC, 2002). EPA has predicted that cumulative pollutant loading limits for the application rates in Table 3 will be reached after 100 years for agriculture, 55 years for forest, 32 years for public contact, and 13 years for reclamation, assuming annual applications (NRC, 2002; U.S. EPA, 1992). Applications are assumed to cease when cumulative loading limits are reached. Time to reach cumulative loading limits assumes a maximum allowed concentration at a maximum application rate. In reality, applications may be more sporadic. Problem formulation may consider actual application rates if available, rather than predicted maximum rates. For example, land application data by county is available from the following seven States: Colorado, Florida, Maryland, New Jersey, New York, Virginia, and Wisconsin (U.S. EPA, 2002c). This group of states includes a large importer of biosolids (Virginia); a large exporter of biosolids (New York); and a State to which EPA has delegated the biosolids program (Wisconsin).

TABLE 3

Estimated Biosolids Application Rates for Different Land Uses

Land Use	No. Observations	Mean Application Rate (metric tons/ha/yr of dry wt)	Standard Deviation	75th Percentile (metric tons/ha/yr of dry wt)
Agriculture	87	6.8	105	16
Forest	2	26	26	34
Public Contact	11	19	122	125
Reclamation	7	74	148	101

Sources: NRC (2002) and EPA (1992).

3.2.3. The Timing of Land Applications of Biosolids

The timing of land applications of biosolids is another factor that determines exposure. In agricultural operations, application is scheduled around tillage, planting, and harvesting, and is also influenced by the physical properties of specific crops, climate regimes, and soil factors (Evanylo, 1999). The State of Virginia recommends that biosolids applied to land between fall and spring have a vegetation cover to minimize the runoff of pathogens and nutrients, and the erosion of sediment-bound biosolids (Evanylo, 1999). However, spray irrigation is not recommended for applying biosolids to forage, row crops, or young tree stands during the growing season, because adherence to leaves can reduce photosynthesis (Evanylo, 1999; McFarland, 2000). Workers who apply biosolids may tend to avoid periods of rain, because their vehicles and equipment may compact or create ruts in soils that reduce crop yields (Evanylo, 1999).

Although rain may be avoided when applying biosolids, literature reviewed for this document did not reveal whether heavy winds might be similarly avoided. Meteorology should certainly be considered when modeling the transport of land-applied biosolids.

3.2.4. Regional Application Issues

Exposure factors that vary by region include: methods of biosolids application, climate, soils, and land available for application in juxtaposition to human populations. A few regional differences in application methods and timing have already been described too in previous sections. Climatic differences contribute to differences in fate and transport of pathogens in biosolids and biosolids-amended soil. For example, pathogen survival tends to be highest in cool, moist soils, such as those in the northeastern United States (Pepper et al., 1993). Hot, dry soils as in the southwestern United States contribute to pathogen mortality (see section below on fate and transport of pathogens). Deficits in rainfall may be counteracted by irrigation in drier climates. Groundwater contamination by pathogens from biosolids is most likely in coarse-textured, sandy soil or land underlain by high permeability karst (NRC, 2002).

The number of people potentially affected by pathogens in biosolids also varies regionally. Potential exposure at the human population level increases as the density of people increases because of greater sewage sludge output, greater needs to find land application sites, higher rates of biosolids applications, and more residents and children potentially exposed near their homes and schools. In the arid southwestern United States, farms are often located far from cities, so fewer residents would be expected to be exposed to pathogens in biosolids (NRC, 2002). However, members of small rural communities closer to urban areas where biosolids are generated and then applied rurally, may have higher individual exposures.

3.3. FATE AND TRANSPORT OF PATHOGENS

3.3.1. Pathogen Survival, Growth and Death

Unlike chemical hazards, biological hazards have the potential to reproduce or to die. Thus, conceptual models need to consider factors affecting the survival and growth of pathogens in biosolids, biosolids-amended soils, and bioaerosols (see Figure 3). Environmental factors affecting the survival of viruses, bacteria and protozoa are presented in Table 4 (Bujoczek et al., 2001; Gerba et al., 2002; Pepper et al., 2006; NRC, 2002). Most enteric pathogenic bacteria are nonspore-formers and relatively sensitive to environmental factors such temperature, desiccation and ultraviolet exposure. Some fecal bacteria have been found to be persistent in biosolids (Vilanova and Blanch, 2005). *Salmonella*, *E. coli* and fecal coliforms are capable of regrowth in moist conditions following treatment (Lang and Smith, 2007; Lang et al., 2007). Regrowth of pathogens can occur in Class A biosolids where biological competition is low compared to Class B biosolids (Zaleski et al., 2005a). However, Zaleski et al. (2005b) demonstrated that pathogens (*Salmonella* and indicators) decreased in numbers when soil was amended with biosolids. Yates and Yates (2007) observed that *E. coli* was able to grow in soil columns with spiked biosolids at 22°C, and bacteriophage phi X174 exhibited the greatest potential for soil transport. More transport was observed in sand rather than loam soils.

Pathogen survival and reproduction are depicted in Figure 3. Note that temperature and moisture are the primary variables affecting the survival of enteric

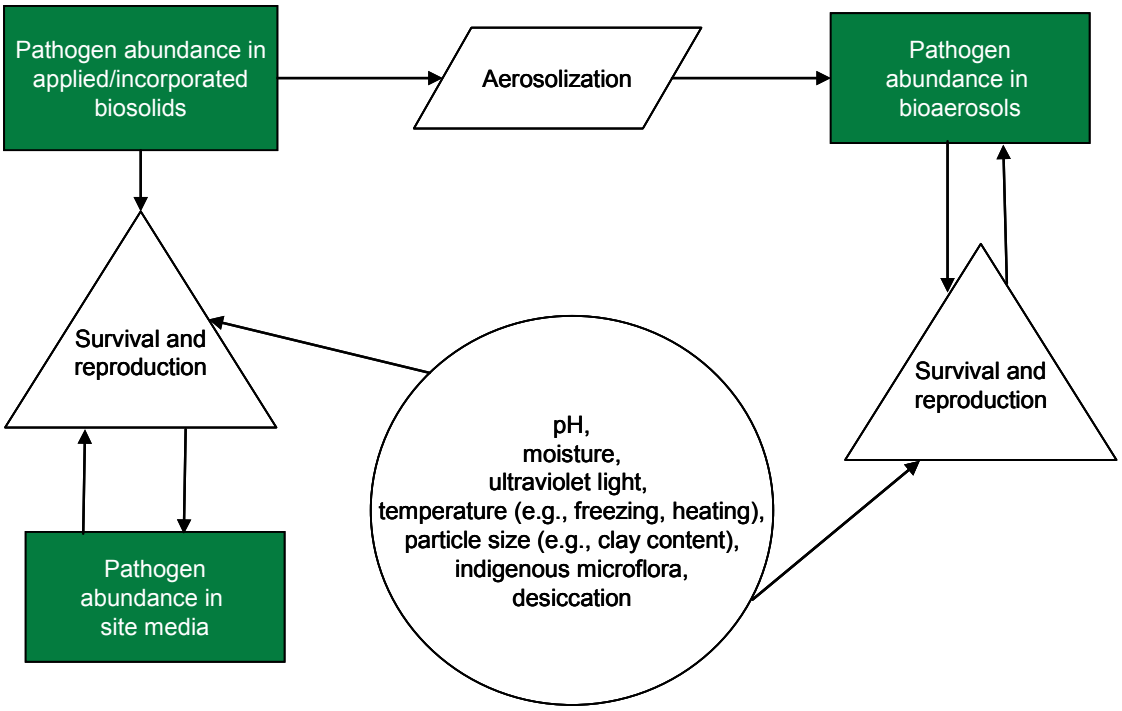

FIGURE 3

Pathogen Fate Conceptual Model

Legend: Green rectangles: sources of pathogen, white parallelograms: natural processes, white triangles: pathogen response (may be positive or negative), white circle: physical environment factors that affect the fate of pathogens.

TABLE 4

Environmental Factors Positively or Negatively Affecting the Survival of Pathogenic Microbes

Parameter	Survival Time		
	Virus	Bacteria	Protozoa
Temperature Increasing	–	–	–
Soil Moisture Decreasing	–	–	–
Rate of Desiccation Increasing	–	–	–
Clay Content Increasing	+	+	Not known
pH range of 6−8	+	+	+

Sources: NRC (2002), Pepper et al. (2006).

viruses in soil (Gerba et al., 2002). In addition to the mechanisms in Table 4, ultraviolet light has the potential to attenuate pathogens, especially those that have been aerosolized (Paez-Rubio and Peccia, 2005; Pepper et al., 2006). Viruses vary considerably in their ability to survive outside a host organism. *Ascaris* eggs may survive several years in soils that are not very wet or very dry (NRC, 2002). Little is known about the viability of protozoa following land application of biosolids (NRC, 2002). Even less is known about the survival and reproduction of pathogens in bioaerosols than about their survival in biosolids or biosolids-amended soil. For more information about the fate of emerging pathogens, a study by Yates and Yates (2007) may be consulted.

3.3.2. Pathogen Transport

Pathogens may be transported from biosolids through various environmental media such as air, soil, and water. In addition to the application process, storage, site-to-site transportation, and loading and unloading are human processes that could mobilize pathogens for transport (see Figure 2). Several mechanisms of transport are possible: aerosolization followed by aerial transport and deposition, erosion, surface runoff and leaching to surface and ground water resources (see Figure 2).

3.3.2.1. *Aerial Transport*

Yates and Yates (2007) observed no microorganisms in air samples collected in a field setting where biosolids were spiked with pathogens (*E. coli, Clostridium perfringens* spores, *Listeria innocua* and bacteriophage phi X174) and applied to monitored soil columns. However, the land application of biosolids may generate bioaerosols either through agitation of the soil during application or following a series of weathering events of deposited biosolids in association with specific climatic conditions (see hazard characterization). Biosolids left on the soil surface or lightly incorporated may be subjected to conditions that lead to drying of the material, rendering it friable. Particulates generated from the friable material are capable of becoming airborne along with the associated pathogens. Bioaerosol droplets or particles may also be generated at the site of biosolids application, storage, site-to-site transport, or loading and

unloading processes, including shoveling biosolids from one pile to another (Straub et al., 1993; Pillai, 2007; Brooks et al., 2007a; Figure 2). Such bioaerosols may potentially move to downwind locations. Wind can erode and resuspend biosolids previously applied to the soil surface (see Figure 2). In contrast, injection of biosolids is typically a barrier to the aerosolization of biosolids (Smith et al., 2005a, Figure 2).

The disking process, one example of "incorporation in soil" in Figure 2, can be a "substantial source of biosolids-derived aerosols" (Paez-Rubio et al., 2006). The emission rate of pathogens during disking of biosolids may be greater than rates during the spreading of dewatered biosolids by side slinger or spraying liquid biosolids (Paez-Rubio et al., 2006). Aerosol emission rates from dewatered biosolids may be higher than those for liquid biosolids (Paez-Rubio et al., 2007). In one study, loading and unloading operations were responsible for the highest predicted annual risks of infection by Coxsackievirus A21 at a distance of 30.5 m (Brooks et al., 2005b).

The launch patterns of bioaerosols from localized sources of biosolids have a conical dispersion form, whereas bioaerosols originating from more spatially extensive fields have a particulate-wave type of dispersion (NRC, 2002; Tanner et al., 2008). Both the application and incorporation processes, as well as site-to-site transport provide moving sources of aerosols. In addition to the source, the physical properties of aerosols and environmental settings affect the dispersal and settling of bioaerosols. Physical properties include the size, density, and shape of droplets or particles. Precipitation, relative humidity, temperature, and air currents can affect dispersal and deposition of aerosolized biosolids (Pillai, 2007).

Evidence from Tanner et al. (2005) suggests that under some conditions, aerosolized viruses may be transported farther than aerosolized gram-negative bacteria, which would affect relative exposure patterns.

3.3.2.2. *Erosion and Runoff to Surface Water*

Water-borne exposure to pathogens from biosolids is driven by precipitation sufficient to move the organisms from the site of application to surface water as runoff (NRC, 2002). The movement of pathogens associated with applied biosolids to surface water depends on the numerous environmental properties of the area where the

biosolids are applied as well as those of adjacent lands. The NRC noted that EPA did not adequately consider the potential for contamination of neighboring properties or surface water by runoff in the Part 503 rule (NRC, 2002). Smith et al. (2005b) identified the monitoring of pathogens in runoff from land application of biosolids to be a research priority, because little is known about this transport pathway.

Where biosolids are applied to the soil surface, runoff may transport particles to surface waters down-gradient (Straub et al., 1993), at least "in principle" (NRC, 2002). Disking operations also break up and mix the biosolids with soil, which increases the potential for erosion and runoff but buries the amendment and dilutes the initial numbers of pathogens. Selvaratnam and Kunberger (2004) reported evidence of a correlation between farmland (amended with treated sewage sludge) runoff and higher incidence of antibiotic resistance and fecal coliforms in downstream surface water. Runoff of pathogens to surface water is expected to be higher where the biosolids are left on the surface (e.g., in forests) compared with incorporation into agricultural soils. Edmonds (1976) found that sewage sludge applied to forest land was unlikely to contaminate groundwater or nearby springs and rivers through vertical movement of the bacteria through the soil. However, the study also indicated that stormwater runoff from forest-applied sludge could potentially contaminate surface waters.

3.3.2.3. *Leaching to Groundwater*

Following precipitation, microorganisms may infiltrate soil and contaminate groundwater (Straub et al., 1993). The NRC noted that EPA did not adequately consider the potential for contamination of groundwater by runoff in the Part 503 rule (NRC, 2002). The transport of microorganisms through soils is affected by both abiotic and biotic factors, including the presence of biosolids, soil characteristics, water flow rates, adhesion processes, filtration effects, the physiological state and mobility of pathogen cells and predation (NRC, 2002). Viruses have a greater potential to be transported to groundwater than other pathogens, although sorption to colloids and biosolid particles limits this potential (NRC, 2002; Chetochine et al., 2006). Transport of larger organisms (bacteria, protozoa, helminths) is less likely, but possible if flows occur through cracks or macropores of soils (NRC, 2002). Transport of pathogens to

groundwater is most likely where soils are sandy and coarse-textured or where karst topography is present (NRC, 2002). Eisenberg et al. (2008) predicted that human health risks due to groundwater exposure are lower than risks from exposure to aerosolized biosolids. Studies that examine pathogen occurrence in groundwater (Borchardt et al., 2003) and survival and inactivation of pathogens in groundwater are available (John and Rose, 2005).

3.3.2.4. *Sorption to Crops*

Pathogens from biosolids could become sorbed to root crops with particles from the biosolids-soil mixture (see Figure 2). Although crops are generally washed before eating, a fraction of biosolids-amended soil will remain sorbed to the crop (estimated at 10% by Gale [2005b]). Additional pathogens might become sorbed to root crops following runoff from biosolids-amended fields to neighboring fields. Leaf crops might become contaminated with pathogens deposited from bioaerosols or from rain splash (see Figure 2). Leaf or root crops could become contaminated with pathogens via irrigation with contaminated surface water or groundwater (see Figure 2). Exposure to contaminated crops is discussed in Section 3.4.2.

3.3.3. Vector Transport

The transport of pathogens from biosolids by insects, birds, pets, and other vectors is possible. For example, flies might become contaminated, leaving trace pathogens on food that is ingested by humans (Graczyk et al., 2001). This potential pathway is included in Figure 2. No information is available on the extent to which land application of biosolids attracts flies or other potential vectors, such as mosquitoes or birds (NRC, 2002). Pets are a potential vector, resulting in dermal, oral (hand to mouth) or respiratory exposures. It is unclear whether procedures in the Part 503 rule that are intended to discourage vectors are effective or not (NRC, 2002). Similarly, it is unclear whether vectors are involved in the transmission of pathogens to humans from land-applied biosolids (NRC, 2002).

3.4. HUMAN ROUTES OF EXPOSURE

Potential routes of exposure to pathogens originating in biosolids include inhalation, ingestion, and dermal exposure (see Figure 2). Problem formulation might consider these exposure routes in the context of bioaerosols or water that come in contact with biosolids, or direct contact with land applied biosolids after the time period required by Class B regulations. Potential exposures associated with noncompliant behaviors are not covered in this document, but could be considered by appropriately modifying or adapting the conceptual models presented here for other scenarios of interest.

3.4.1. Inhalation

Routes of exposure for aerosolized pathogens are not well characterized but likely involve a combination of both inhalation and ingestion pathways (Pillai, 2007, Figure 2). Pathogens can be physically transported away from the site of land application on aerosolized particles of biosolid material. Large aerosolized particles (between 5 and 20 μm) can deposit in the upper respiratory tract. Clearance of these particles results in oral exposures. Smaller particles penetrate deep into the lungs and may be lodged and retained by the alveoli (Pillai, 2007). Several reports of respiratory symptoms associated with biosolids application (e.g., Herr et al., 2003; Gavett and Koren, 2001; George et al., 2001) indicate inhalation is the most probable route of exposure to smaller particles. In one study that investigated bioaerosols emitted during the spreading of dewatered Class B biosolids onto farm land, the diameters of most emitted particles were of inhalable and possibly respirable size (Paez-Rubio et al., 2007). Because of the high volume of air that is inhaled daily, Pillai and Ricke (2002) assert that inhalation is the predominant route of exposure for aerosolized pathogens that may result in adverse health effects. It should be noted that not all pathogens can infect tissues exposed during inhalation.

The NRC (2002) determined that the inhalation pathway was among the routes of exposure that was not adequately assessed by EPA in the development of the Part 503 rule. They noted that inhalation of dust was presumed by EPA to occur only on-site and that controlling site access was thought to prevent that route of exposure

(NRC, 2002). Brooks et al. (2005a,b) investigated the potential for inhalation of pathogens by off-site residents. However, the literature search did not locate many studies of inhalation of biosolids-derived aerosols or pathogens by off-site residents. Thus, inhalation of pathogens by off-site residents needs more consideration. For more information about the potential health effects of inhalation hazards, see Shusterman (1992) and Lewis et al. (2001).

3.4.2. Ingestion

Ingestion of biosolids-related pathogens may occur via several exposure scenarios including; direct and incidental ingestion of surface or groundwater containing pathogens originating from biosolids; ingestion of pathogens which are sorbed to crops and food items after application of biosolids in agricultural fields; incidental ingestion of pathogens associated with surface-applied biosolids and biosolids mixed with soil; and ingestion of bioaerosols containing pathogens (see Figure 2).

Ingestion of biosolids in soil occurs through the transfer of pathogens to the mouth from contaminated hands or crops or though inhalation followed by swallowing (Gerba et al., 2002; Figure 2). Larger particles in contact with the respiratory tract can be cleared from the tract and swallowed. Researchers vary in their estimation of the percentage of inhaled pathogens ingested (Pillai, 2007).

Ingestion of groundwater or surface water is a potential route of exposure to biosolids-derived pathogens. Untreated surface water contaminated with pathogens from biosolids might be ingested while swimming, potentially allowing for greater consumption of pathogens than a domestic tap supplying water treated in accordance with the Safe Drinking Water Act (SDWA).

Food consumption is a potential direct route of exposure to pathogens, especially involving ingestion of foods not subjected to cooking or washing. Biosolids are applied to agricultural soil to improve its fertility and to enhance crop yields. The application of biosolids to soil along with consumption of food grown on amended fields provides an avenue of exposure to pathogens through the food chain. Reasonable exposure scenarios involve the adherence of the pathogens to the plant (i.e., roots, stems,

leaves), particularly the edible portion of the plant, and subsequent consumption by individuals.

At least three exposure scenarios may result in the ingestion of pathogens associated with biosolids when applied in crop settings. Each exposure scenario differs with respect to the portion of the plant that is eventually consumed. The first scenario involves the deposition of aerosolized material on the surface of the aboveground portions of the plant (see Figure 2). This exposure may arise during biosolids application. In this scenario, biosolids may be applied by spreading or spraying the material onto the soil with the resulting generation of airborne pathogens from the biosolids (see Figure 2). Pathogens and biosolids material subsequently land on and adhere to the aboveground portion of the plant that is intended for consumption. Compliance with current regulations makes pathogen ingestion from crops a more unlikely exposure pathway for farm residents (see the section on regulatory restrictions below). Part 503 regulations provide for time restrictions between application to the field and harvesting of plants (see Table 5). However, harvesting of plants in nearby fields where pathogen deposition from air or surface runoff may occur is not restricted. Additionally, the placement of microorganisms on the aboveground portion of the plant subjects the pathogens to environmental stressors such as UV radiation and desiccation, both of which diminish the viability and spread of the pathogens. Moreover, the types of foods that may be affected by the deposition of aerosolized material are grains and some vegetables which normally undergo preparation and cleaning processes to reduce pathogen viability prior to consumption. Although this scenario might constitute a minor pathway, it should be considered in a problem formulation if appropriate.

The second exposure scenario associated with crops addresses plant consumption in which the palatable portion is aboveground, but is expected to come in contact with the soil. This scenario includes some fruits and vegetables such as melons, cucumbers, and tomatoes. This scenario allows for extended contact with soil while the plant develops with the possibility of infection of the plant through a lesion or by adherence to the plant surface. Many of the crops that fall into this category include vegetables that are consumed without prior food preparation other than normal

TABLE 5

Pathways of Exposure and Applicable Use Restrictions for Class B Biosolids

Pathways	Part 503 Required Use Restriction
Handling soil from fields where biosolids have been applied	No public access[a] to application until at least 1 year after Class B biosolids application
Handling soil or food from home gardens where biosolids have been applied	Class B biosolids may not be applied on home gardens
Inhaling dust[b]	No public access to application sites until at least 1 year after Class B biosolids application
Walking through fields where biosolids have been applied[b]	No public access to fields until at least 1 year after Class B biosolids application
Consuming crops from fields on which biosolids have been applied	Site restrictions that prevent the harvesting of crops until environmental attenuation has taken place
Consuming milk or animal products from animals grazing on fields where biosolids have been applied	No animal grazing for 30 days after Class B biosolids have been applied
Ingesting surface water contaminated by runoff from fields where biosolids have been applied	Class B biosolids may not be applied within 10 meters of any waters to prevent runoff from biosolids-amended land
Ingesting inadequately cooked fish from water contaminated by runoff from fields where biosolids have been applied, affecting the surface water	Class B biosolids may not be applied within 10 meters of any waters to prevent runoff from biosolids-amended land
Contact with vectors that have been in contact with biosolids	All land-applied biosolids must meet one of the vector-attraction-reduction options

[a]Public-access restrictions do not apply to farm workers. If there is low probability of public exposure to an application site, the public-access restrictions apply for only 30 days. However, application sites that are likely to be accessed by the public, such as ballfields, are subject to 1-year public-access restrictions.

[b]Agricultural land is private property and not considered to have a high potential for public access. Nonetheless, public-access restrictions are applied.

Source: Taken from NRC (2002), which adapted the table from EPA (1999).

washing, which may not apply to all households. However, as the area of contact is with the soil surface, it is anticipated that the pathogens would be exposed to higher levels of environmental stressors which would reduce the viability of pathogens. The third scenario applies to crops that have the palatable portion below the soil surface. Examples include root vegetables, such as potatoes, carrots, and yams. This scenario poses a concern for several reasons. First, this exposure scenario involves direct contact to pathogens with the greatest potential for long-term survival, i.e., those that are found below the soil surface. Furthermore, because the food portion of the plant develops in close contact with the soil, it has the greatest potential for retaining pathogens on the plant surface (Chale-Matsau and Snyman, 2006). Finally, some tubers may be ingested with little or no preparation that would remove or inactivate pathogens on the edible plant surface. For example, carrots are usually eaten raw. They may be washed or skinned prior to eating, but the amount of preparation varies considerably.

Part 503 regulations address these exposure scenarios for Class B biosolids through appropriate grazing, harvesting, and public access restrictions. Existing regulations establish temporal restrictions on the harvesting and consumption of food grown on land receiving Class B biosolids. Planting is indirectly restricted because the harvesting restrictions are 30 days to 38 months, depending on the part of the plant that is harvested, and some crops require less than 38 months from planting to harvesting.

Nonetheless the potential remains for consuming food harvested from amended plots. As presented in the section on regulatory restrictions (below), Part 503 regulations require a waiting time of either 20 or 38 months for crops whose harvested portion is below ground; and shorter periods for crops where the above-ground portion is harvested. Pathogens capable of surviving over this period of time can adhere to the surface of the harvested portion of the plant, and with inadequate food preparation steps, could be consumed.

3.4.3. Dermal Exposure

Dermal contact constitutes a direct method of transfer of pathogens in biosolids to receptors (see Figure 2). Dermal transmission of pathogens would occur primarily

through skin abrasions or cuts, either through contact with contaminated soil or surface water.

Dermal contact may also occur during occupational exposure or during unintended contact with biosolids that have moved from the original site of application (e.g., through aerial dispersion or runoff). Workers most likely come in contact with biosolids when processing, loading, and applying them.

Recreation during the summer months may be another possible exposure scenario to consider. For example, swimming might permit dermal contact (as well as the ingestion or inhalation) of pathogens in surface waters contaminated by biosolids applied to the surrounding landscape. To assess dermal exposures, the risk assessor would need information on the amount of material adhering to the skin and dose-response values for the pathogens of interest, as well as data, on the distribution and numbers of pathogens in biosolids and their potential for regrowth or reproduction.

3.5. REGULATORY RESTRICTIONS

Many site restrictions related to land application of biosolids are intended to reduce exposure to pathogens and chemicals in the material (see Table 5). As such, these restrictions will affect the plausibility of various exposure pathways in a particular conceptual model. Time intervals required prior to site access are summarized in Table 6. Particular states may also have varying regulatory criteria for: required distances between land-applications and surface waters or wetlands, slope restrictions, depths to groundwater and bedrock, soil permeability rates, distances to residences, schools, health care facilities or recreation areas, and distances to private or public water-supply wells (NRC, 2002).

3.6. FACTORS THAT AFFECT INFECTION AND DISEASE

Several host and pathogen characteristics affect the probability or intensity of disease (see Figure 4). For planning of a specific risk assessment, attributes of pathogens need to be considered together with attributes of the host and environment. For example:

TABLE 6

Minimum Time Interval between Application and Harvest, Grazing or Public Access to Lands Applied with Class B Biosolids

Criteria		Injection	Surface Application	Surface With Incorporation
Harvest	Food crops whose harvested parts may contact biosolids-amended soil	14 months	14 months	14 months
	Food crops whose harvested parts grow in soil	38 months	20 or 38 months*	38 months
	Food, feed and fiber crops	30 days	30 days	30 days
Grazing	Animal grazing	30 days	30 days	30 days
Public Access	High potential for exposure	1 year	1 year	1 year
	Low potential for exposure	30 days	30 days	30 days

*The 20-month interval prior to harvesting applies if the biosolids stay on the surface for 4 months or longer prior to incorporation. The 38-month interval applies if the biosolids stay on the surface for less than 4 months prior to incorporation.

Source: Modified from: NRC (2002) and 40 CFR Part 503.

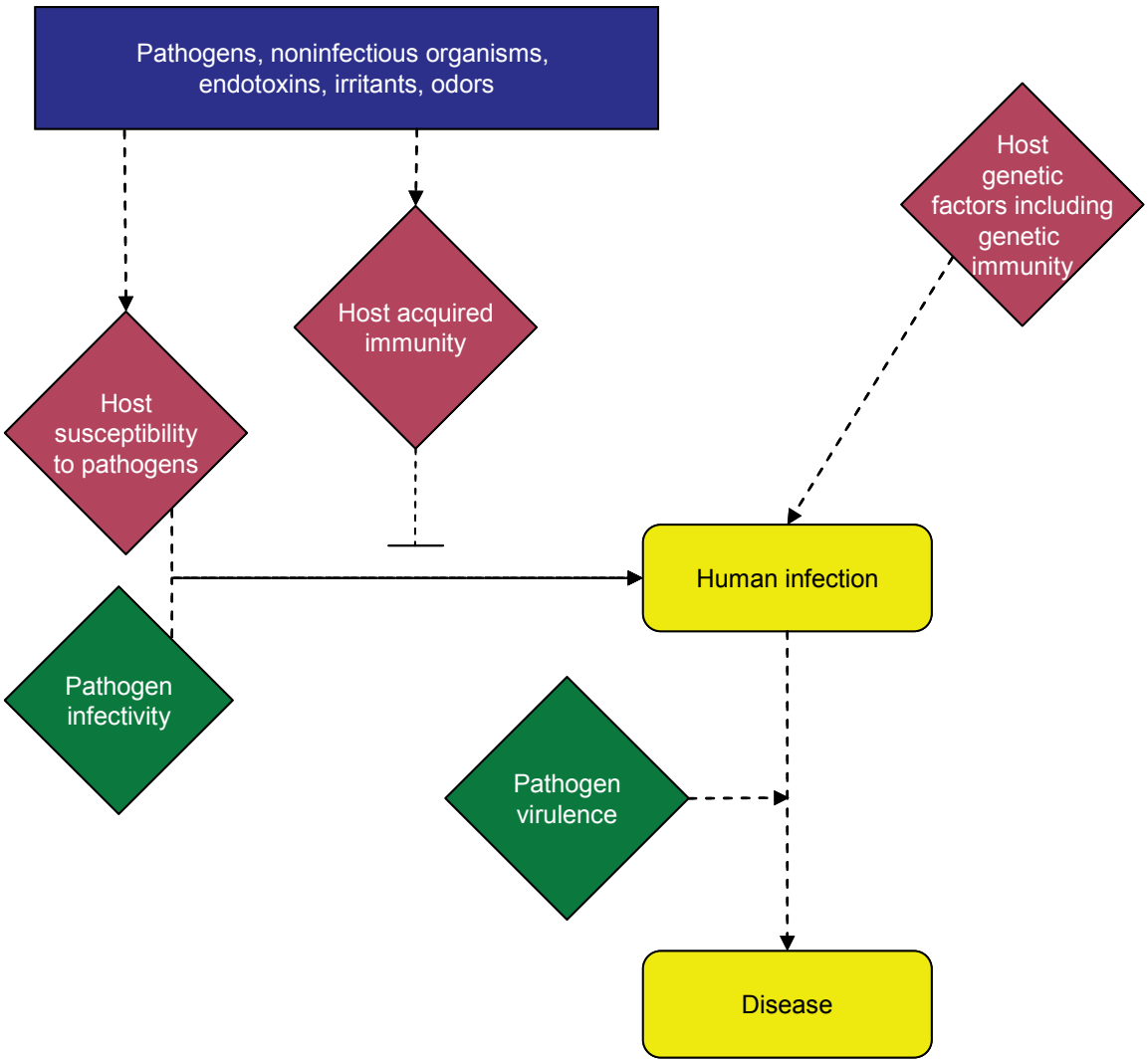

FIGURE 4

Disease Factors Conceptual Model

Legend: Blue box: hazards; purple diamonds: host factors; green diamonds: pathogen factors; yellow rounded squares: host outcomes; solid lines: “leads to”; dashed lines: “influences”; T-line: “inhibits.”

1) Host immunity may be important and differs for different pathogens

2) Person to person transmission can be an aspect of a particular setting within which biosolids are being applied and in those cases can be included in the risk assessment

3) Human activities (see Figure 2) can affect biosolids and the differences in locations between generation and application can be important. Thus, "community factors" are a potentially important aspect of assessing risks associated with applications of biosolids.

Other key human and pathogen factors are described below.

3.6.1. Human Factors

Three host factors discussed in NRC (2002) are concomitant exposures, genetic factors and acquired immunity. Based on ILSI (2000), the *Draft Protocol for Microbial Risk Assessment to Support Human Health Protection for Water-Based Media* (U.S. EPA, 2009a) includes the following host characteristics. Each has the potential to influence exposure and health effects (U.S. EPA, 2009a):

- Immune status (also see Section 3.6.1.3)
- Age (also see Section 3.6.2)
- Concurrent illness/medical treatment
- Genetic background (also see Section 3.6.1.2)
- Pregnancy
- Nutritional status
- Previous exposure
- Social/behavioral traits

Each of these factors may impact a risk assessment concerning host susceptibility to disease and severity of illness, and would be useful for consideration on a case-by-case basis. Differential susceptibility within the population may be explicitly, implicitly, or not considered. Transparency is enhanced when risk assessors can document how and why each host factor is considered or not.

3.6.1.1. *Concomitant Exposures*

Various hazards such as pathogens, noninfectious organisms, cellular components, irritants, and odors may influence individual immunity, other aspects of susceptibility, or the nature or intensity of disease (see Figure 4; Schiffman et al., 2000). Synergistic effects might result from combined exposures to these hazards (NRC, 2002, Figure 4). For example, endotoxins may combine with particles and allergenic components to promote the development of respiratory diseases and systemic effects (NRC, 2002).

3.6.1.2. *Genetic Factors*

Genetic factors influence individual immunity as well as other aspects of disease susceptibility (see Figure 4). Genetic factors such as a predisposition to asthma attacks can be a factor in determining whether infection proceeds to disease (Bracken et al., 2002). No information is available on the role of genetic factors in contributing to health effects due to bioaerosols from land-applied biosolids (NRC, 2002).

3.6.1.3. *Acquired Immunity*

Acquired immunity is the result of previous exposure to pathogens and is part of the immunity box in Figure 4. Acquired immunity can reduce the fraction of illness in a population exposed to pathogens (NRC, 2002). Genetic factors also contribute to the immune status of an individual. The dynamics of immunity are not well understood for most pathogens. Loss of immunity to pathogens is also a possible result of exposure to other pathogens, or biological or chemical hazards (see Figure 4).

3.6.2. Additional Susceptibility Factors

For public health risk assessment purposes, exposed populations are evaluated based on age (children, adults, geriatrics). In addition, sensitive subpopulations may be evaluated based on gender, ethnicity, baseline health status (immunocompromised, hereditary diseases, etc.) or any other site-specific health characteristic of the potentially exposed population that warrants special consideration.

3.6.3. Pathogen Factors

Infectivity and virulence are pathogen factors that can also influence infection and disease (see Figure 4). Infectivity is the relationship between the quantity of pathogens ingested or inhaled or in contact with skin and the probability of infection. For many pathogens, risk assessors have typically assumed a no-threshold effect (i.e., assumed that one organism is sufficient to produce infection in some portion of an exposed population or subgroup) (Haas et al., 1999, also see Analysis Plan chapter for discussion of thresholds). Virulence is a measure of the severity of the disease that the pathogen is capable of causing.[5]

3.7. INFECTION AND DISEASE

Two primary, broad endpoints of risk assessments for pathogens in land-applied biosolids are human infection and disease (see Figures 2 and 4−9). Infection is the process by which a microorganism multiplies or grows in or on the host. Clinical diseases are evidenced by signs or symptoms. Soller and Eisenberg (2008) provide parameter values for the proportion of infected individuals with symptomatic responses for enteroviruses, rotavirus, *Cryptosporidium*, *Giardia lamblia*, Salmonella, *E. coli* O157:H7, Shigella, and a composite value (minimum of 10, median of 40, maximum of 75%).

A variety of diseases may arise from exposure to enteric viruses (i.e., enterovirus, rotavirus, adenovirus) such as gasteroenteritis, respiratory illness, cardiovascular disease and central nervous system disorders. Likewise, the enteric bacteria associated with biosolids such as *Salmonella*, *Shigella*, C*ampylobacter*, *E. coli* and *Listeria* have been identified as causative agents of illness in exposed humans. Infections of enteric bacteria have resulted in gastrointestinal illness, dysentery, arthritis, Reiter and Guillain-Barre syndrome, and neuromuscular paralysis (see Table 2). The protozoans of concern *Giardia*, *Cryptosporidium* and *Entamoeba*, produce cysts and oocysts which have been shown to be environmentally stable and somewhat resistant to disinfectants. Thus, they are recognized as significant human pathogens with the

[5] Some authors include infectivity in the definition of virulence, but in this context virulence is separate from infectivity. For further discussion of risk assessment terminology see EPA (2007).

potential to cause gastrointestinal illness exhibited by diarrhea, dehydration and weight loss and, in the case of *Cryptosporidium* and immunocompromised individuals, mortality too. Potential effects of particular pathogens found in biosolids are described in the hazard characterization chapter (see Section 2.2).

Public health endpoints may include the prevalence (total number of cases in a population) or incidence (number of new cases in a population during a specific time interval) of disease (or morbidity). For example, severity (e.g., number of days lost to illness) may be another property of disease that is of interest to the risk assessor. Mortality is an additional, potential endpoint. For further discussion on issues relating to the severity of illness, see EPA (2009a).

3.8. SCENARIOS

Risk assessors may describe scenarios that do not include all the pathways shown in Figure 2. For example, in this section, five exposure scenarios representing common public concerns are presented to demonstrate how Figure 2 may be modified to address more specific scenarios of interest. These include the following:

1. Neighboring residences and schools adjacent to a site applied with biosolids
2. Residents of a site where biosolids are applied (e.g., farm families)
3. A pica child exposed to biosolids
4. Drinking water consumers of groundwater aquifer supplies underlying sites applied with biosolids (i.e., particularly those with highly permeable soils or shallow water tables)
5. Drinking water consumers of surface waters downstream from sites where biosolids are applied

EPA's Exposure Factor's Handbook supplies exposure estimates for many types of behaviors that may be included in these and other scenarios assessors may investigate (U.S. EPA, 1997).

3.8.1. Scenario 1. Neighboring Residences and Schools

Individuals potentially exposed to biosolids-derived pathogens may reside on lands adjacent to farms, forests, reclaimed minelands, or other lands where biosolids are applied. Similarly, schoolchildren may be exposed to eroded soils or bioaerosols from land-applied biosolids. The conceptual model for this scenario (see Figure 5) adapts most of the pathways from the general conceptual model (see Figure 2). The primary source processes that do not appear in this scenario are storage, transport and loading and unloading activities (see Figure 5). For this example it is assumed that the biosolids were stored, loaded, and unloaded in an enclosed facility, so exposure from these activities need not be addressed. Other scenarios could be developed to assess the loading and unloading of biosolids in open facilities, or cases where leaks or runoff, for example, may occur.

3.8.2. Scenario 2. Residents (Farm Families)

Individuals potentially exposed to biosolids-derived pathogens may reside on farms where biosolids are applied. The conceptual model for this scenario (see Figure 6) adapts all of the potential pathways from the general conceptual model including storage, transport and loading and unloading activities (see Figure 2). However, a specific model for farm families might include pathways by which biosolids-amended soil is tracked into the residence (e.g., contaminated boots, work clothes, or equipment that is returned to the barn). Recreational hikers in forests where biosolids have been applied might also bring pathogens home on their clothing.

3.8.3. Scenario 3. Pica Child

Soil ingestion is the consumption of soil as the result of various behaviors such as consuming soil directly or contacting dirty hands or contaminated crops. Moreover, soil-pica, the scenario considered here, is the recurrent ingestion of unusually high amounts of soil (i.e., on the order of 1–5 grams per day). Groups at risk of soil-pica behavior are generally any child aged 6 years and younger including children within that same age range in farm families or at schools discussed in Scenarios 1 and 2. Noting that soil ingestion is a normal behavior among children, evaluation of all types of soil ingestion is included in the soil-pica scenario (see Figure 7).

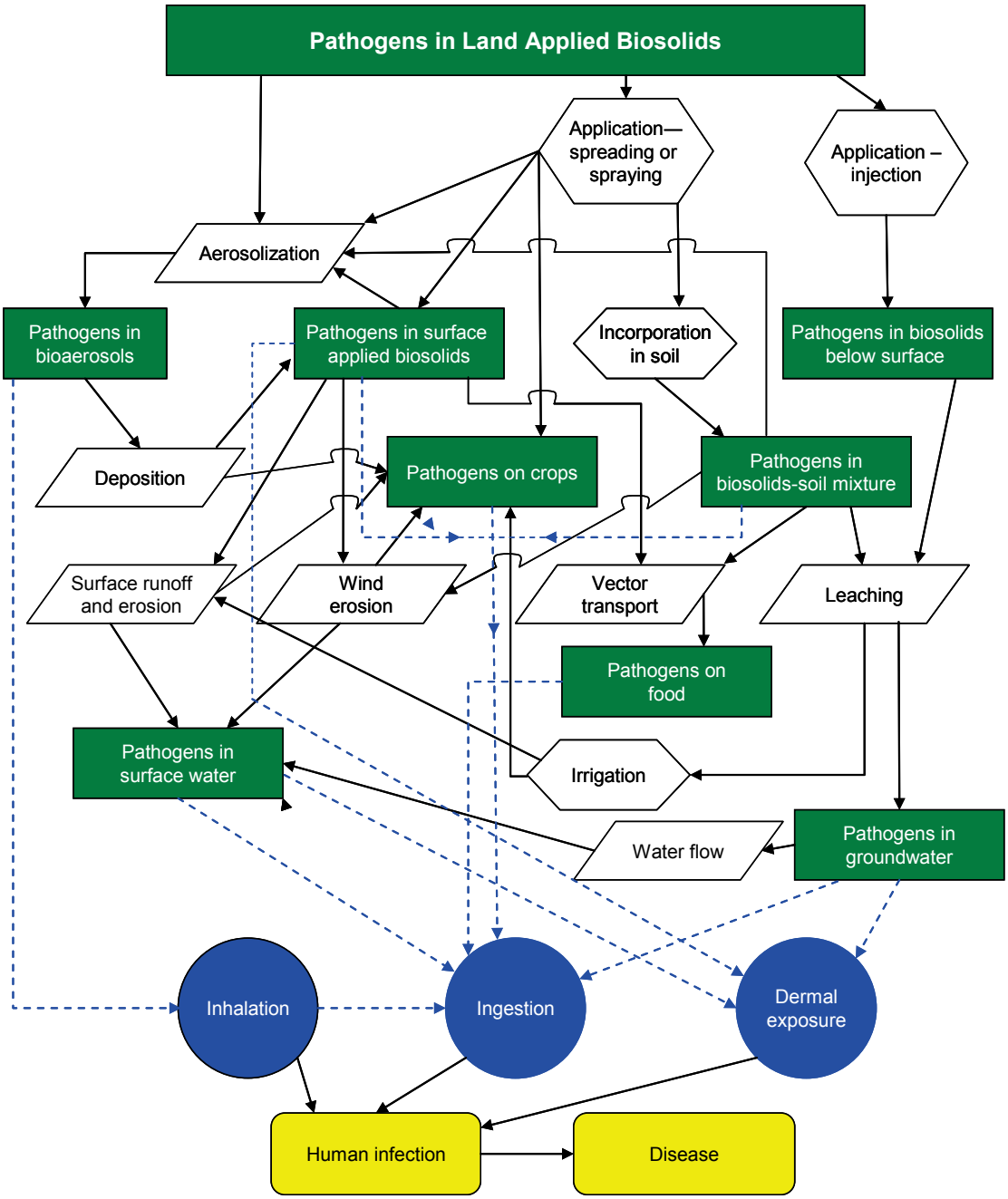

FIGURE 5

Scenario 1: Neighboring Residences and Schools (or Adjacent Property) Conceptual Model

Legend: Green rectangles: sources of pathogen, white hexagons: human actions, white parallelograms: natural processes, blue circles: exposure pathways, yellow rectangles with rounded corners: potential responses.

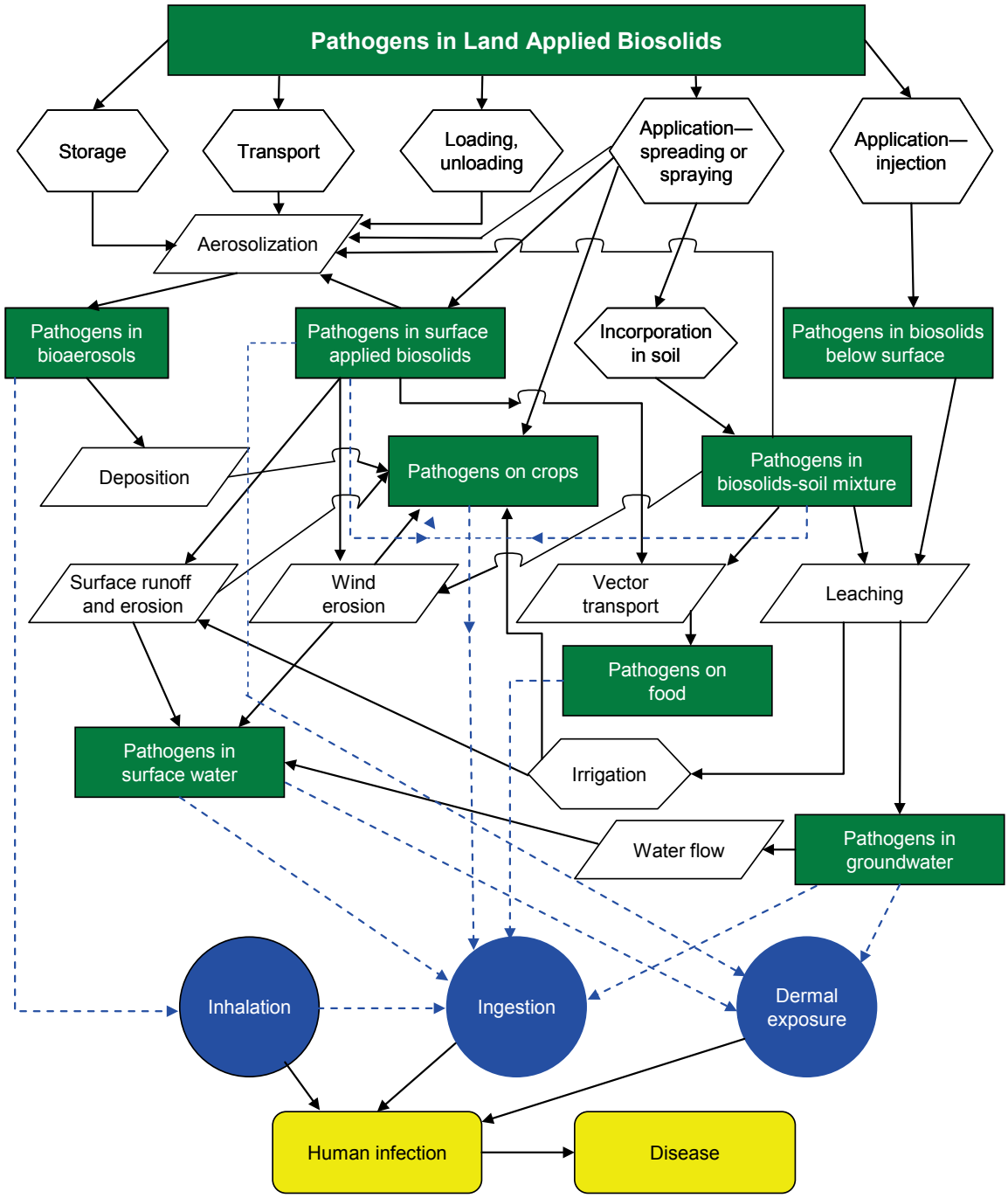

FIGURE 6

Scenario 2: Resident (Farm Family) Conceptual Model

Legend: Green rectangles: sources of pathogen, white hexagons: human actions, white parallelograms: natural processes, blue circles: exposure pathways, yellow rectangles with rounded corners: potential responses.

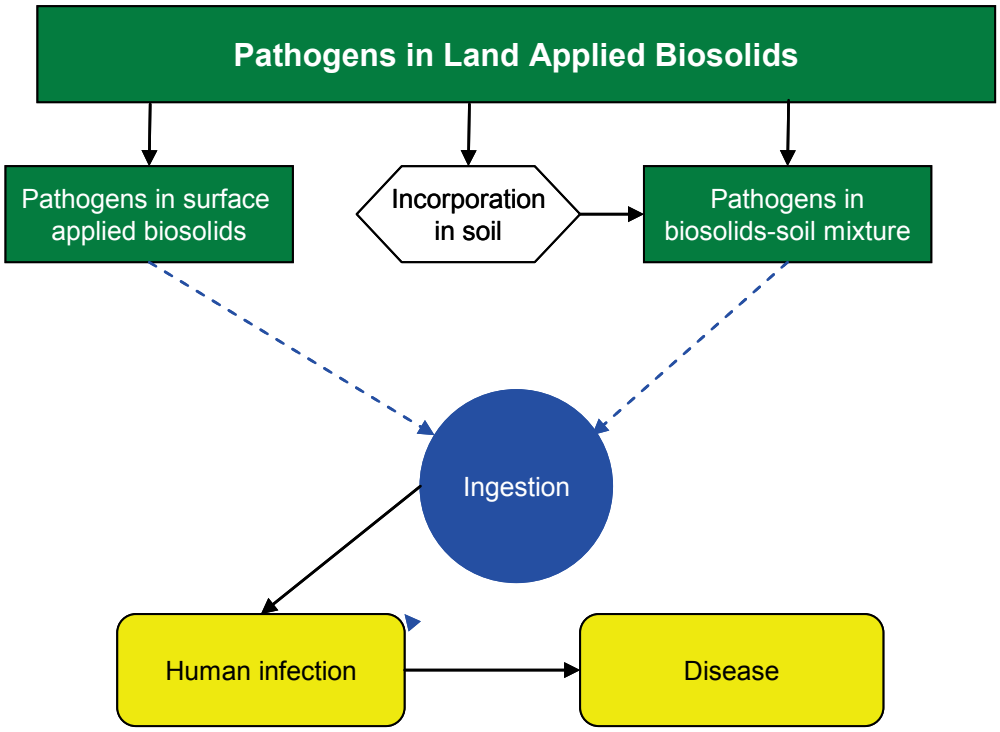

FIGURE 7

Scenario 3: Pica Child Conceptual Model

Legend: Green rectangles: sources of pathogen, White hexagons: human actions, White parallelograms: natural processes, Blue circles: exposure pathways, Yellow rectangles with rounded corners: potential responses.

3.8.4. Scenario 4. Drinking Water Consumers of Groundwater

Leaching of pathogens to groundwater is of potential concern following the injection of biosolids in the subsurface, or after surface applications to porous soils or Karst topography overlying an aquifer or well. Studies conducted on porous soils have demonstrated that pathogens in water can move with the liquid through different vertical horizons in the soil (Chetochine et al., 2006). Groundwater aquifers serve as the sole source of water in many communities and therefore may be used for both farming and domestic purposes. For example, groundwater may be consumed and used in food preparation (e.g., when washing fruits or vegetables or cooking). Cooking, in particular, can account for a significant reduction or elimination of most pathogens. Groundwater is also used in such communities for bathing and other household activities. This scenario emphasizes groundwater consumption (see Figure 8). Risk assessors may choose to consider groundwater sources covered by the SDWA separately from private wells, which are not regulated by the SDWA.

3.8.5. Scenario 5. Drinking Water Consumers of Surface Water

The use of down-gradient surface waters as a source of potable water may result in exposure to biosolids-related pathogens (see Figure 9). The major pathways of potential exposure to pathogens would be erosion of biosolids particles and surface runoff from treated sites (see Figure 9). Additionally, pathogens might be carried to surface water through interactions with contaminated groundwater, or possibly deposited to surface water following aerial transport. Treatment of water before consumption can greatly reduce the potential for exposure pathogens. Water supplies regulated by SDWA require between 3-log and 5-log removal/inactivation efficacies prior to public consumption.[6]

3.8.6. Event Related and Regional Aspects of Scenarios

These scenarios are merely representative and not meant to be exhaustive. Different scenarios will certainly occur in different regions or may be more important to consider depending on the circumstances. For example, surface water drinking

[6] Maximum contaminant levels under SDWA http://www.epa.gov/safewater/contaminants/index.html.

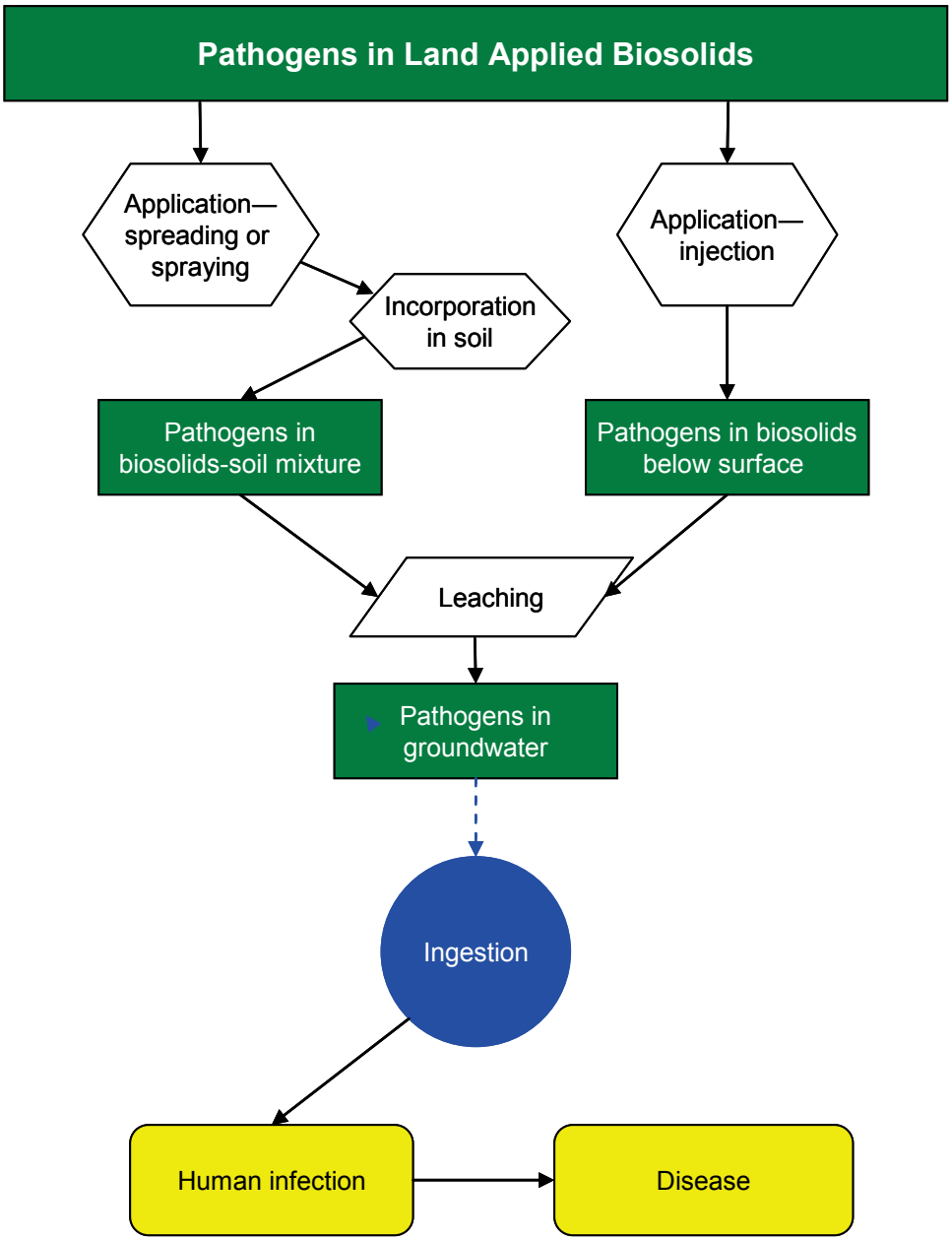

FIGURE 8

Scenario 4: Groundwater Conceptual Model

Legend: Green rectangles: sources of pathogen, White hexagons: human actions, White parallelograms: natural processes, Blue circles: exposure pathways, Yellow rectangles with rounded corners: potential responses.

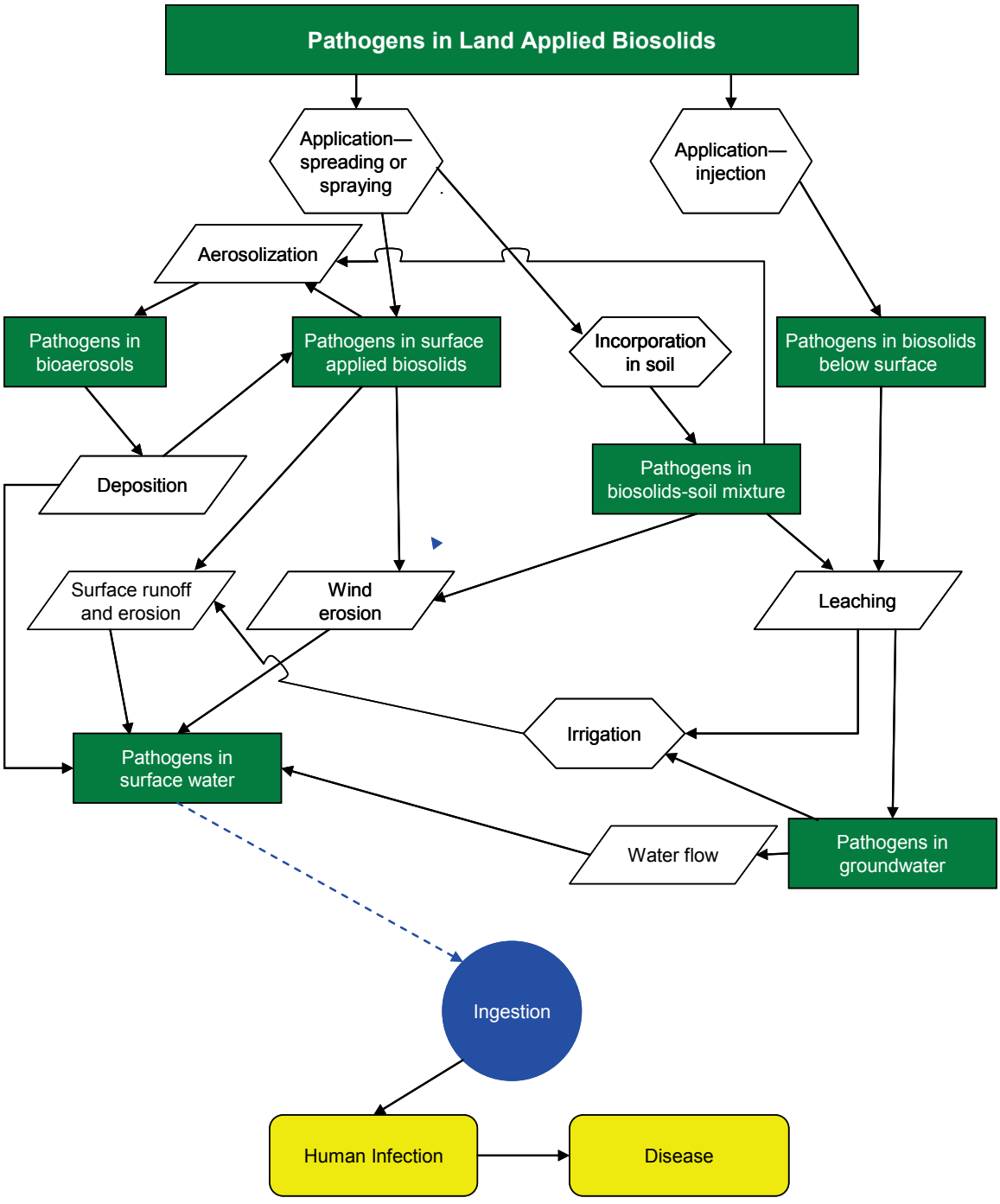

FIGURE 9

Scenario 5: Surface Water Conceptual Model

Legend: Green rectangles: sources of pathogen, White hexagons: human actions, White parallelograms: natural processes, Blue circles: exposure pathways, Yellow rectangles with rounded corners: potential responses.

scenarios would be less applicable to arid regions if application is during the dry season. Scenarios involving aerosolization of pathogens in biosolids would be more applicable to windy regions. Development of region-specific risk assessments and research would benefit from modifying the scenarios based on the relevant circumstances of each individual case.

In addition, risk assessors may want to consider scenarios that include relatively common events, such as storms, or possibly rarer events such as floods, hurricanes, and tornados. For arid regions, seasonal factors may influence runoff and erosion (Moffet et al., 2005). Consideration of the timing of biosolids application may be important if the region has notable wet weather and/or dry weather patterns. As mentioned previously, application of biosolids to frozen ground is not permitted.

4. SCREENING OUT ELEMENTS OF THE CONCEPTUAL MODEL

On a case by case basis, the general conceptual model (see Figure 2) may be examined to determine if sufficient information is available to screen out unlikely hazards, scenarios, routes of exposure, or endpoints from consideration in risk assessments of pathogens in biosolids. This effort should help simplify the model to focus on the most pertinent factors and should not be confused with the screening-level risk assessment process that is site-specific and part of the analysis phase rather than the problem formulation.

Very little information is available to directly compare the relative importance of different exposure pathways. Academic studies tend to emphasize a single exposure pathway rather than a comparison of multiple pathways. However, the literature review (see the Appendix) suggests that certain pathogens and exposure pathways tend to be less likely, for example:

- Endotoxin. Brooks et al. (2007a) found that biosolids-amended soil did not have higher levels of endotoxin than unamended soil. Levels of endotoxin in aerosolized soil were sometimes above those associated with aerosolized, biosolids-amended soil, calling into question whether biosolids were the primary source of the endotoxin (Brooks et al., 2006).

- *Staphylococcus aureus*. A broad study of 15 sites across the United States found that *S. aureus* was detected in raw sewage samples but not in biosolids (Rusin et al., 2003a).

- Certain protozoa. Gerba et al. (2002) determined that microsporidia and *Cyclospora* would not be likely to survive under high temperatures of anaerobic digestion or under conditions of low moisture in Class B biosolids treatment.

- Certain bacterial or viral pathogens in bioaerosols. Pathogens and indicator bacteria were only rarely found in aerosolized samples in a study of land application of biosolids in Tucson, AZ. These included coliforms and coliphages, which were present at high densities in biosolids. The authors suggested that only microorganisms in the aqueous phase of biosolids were able to aerosolize; others remained sorbed to the solid phase (Brooks et al., 2004a). Furthermore, Tanner et al. (2005) determined bioaerosol emission rates and plume characteristics during spray application of liquid Class B biosolids. They did not detect coliphages or coliform bacteria just downwind of the biosolids application, though pathogens sprayed in inoculated groundwater were detected. The

researchers concluded that the presence of biosolids reduces aerosolization of microorganisms relative to application of inoculated groundwater. The duration of exposure to any pathogens (below detection limits) downwind of biosolids application is brief (Tanner et al., 2005).

However, insufficient evidence exists to support any broad generalizations about negligible elements at this time.

Brooks et al. (2005b) undertook a study to estimate risks of microbial infection of residents near biosolids application sites. At 10 sites (five in Arizona, five elsewhere in the United States) amended with either liquid or solid Class B biosolids, they measured heterotrophic plate counts of bacteria, total coliform bacteria, *E. coli*, *Clostridium perfringens*, coliphage, enteroviruses, hepatitis A virus and norovirus in aerosol samples downwind from application sites. The study distinguished between loading, unloading, land application and background operations. Of the microorganisms evaluated, the greatest risk of infection was from coxsackievirus A21 from loading operations, having a 4×10^{-4} chance of infection (Brooks et al., 2005b). Based on this work, Pepper et al. (2006) concluded that the overall community risk of infection from bioaerosols during land application was relatively negligible.

Some evidence (below) might support a decision to screen out certain exposure pathways in Figure 2 from general or regional consideration in the future. However, more evidence is needed to support such a judgment.

- Groundwater pathway. Because of the large size of bacteria, soil (especially fine-textured soil) can act as a filter to limit bacterial transport (NRC, 2002). Soil would also be expected to limit the transport of larger protozoa and helminths (NRC, 2002). A review of the literature has concluded that few pathogens (even viruses) from biosolids leach to groundwater, except perhaps if biosolids are applied over karst topography (Pepper et al., 2006; Edmonds, 1976). Although Gerba (2005) acknowledges that of the pathogens in biosolids, viruses have the greatest potential for contamination of groundwater, Pepper et al. (2006) concluded that "groundwater contamination from land-applied biosolids does not appear to be likely." Sandy soils with low cation exchange capacity deserve more study.

- Root crop ingestion pathway. A United Kingdom study of infection from consumption of root crops grown on biosolids-amended soils found that risks to humans was low. Seven pathogens were included in the study: salmonellas,

Listeria monocytogenes, campylobacters, *Escherichia coli* O157, *Cryptosporidium parvum*, *Giardia* and enteroviruses (Gale, 2005b). However, United Kingdom biosolids may not be comparable to Class B biosolids in the United States.

Regulations might also allow a risk assessor to screen out potential pathways of exposure in the general case. For example, if biosolids must be stored in enclosed facilities, the generation of bioaerosols from that source (and exposure to neighboring residents) would not be likely.

5. CONSIDERATIONS FOR DEVELOPING AN ANALYSIS PLAN

5.1. INTRODUCTION

The analysis plan is the final stage of problem formulation. It summarizes the measures, methods, and data needs for conducting the exposure, health effects and risk assessment steps. Providing a rational for the selection of measures, methods and tools is essential for transparency. All of these should be described in the context of the sources, pathways, environmental media, and human health endpoints described in the conceptual model. The emphasis is on uncertainties and variables to which the risk assessment is sensitive, if known. A rigorous analysis plan is especially necessary if there is no established protocol for conducting a particular type of risk assessment (U.S. EPA, 1998), as with human health risk assessment of biosolids-derived pathogens.

The analysis plan evaluates risk hypotheses to determine how they will be assessed (U.S. EPA, 1998, 2003a). A rationale for selecting or eliminating risk hypotheses has been presented in *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998). An analysis plan for a risk assessment of pathogens in biosolids can be designed to eliminate negligible pathways in the conceptual model (e.g., refer to Chapter 4 of this document). Available data are described, as well as new data that, if collected, might enhance the risk assessment. The feasibility of collecting new data can also be discussed. There are no definitive guidelines on what constitutes adequate data. A transparent description of available data and data needs helps reviewers and users of a risk assessment to understand judgments made. Lack of transparency can be more damaging to a risk assessment's utility than judgments and assumptions that lead to a healthy scientific debate. The analysis plan addresses both measurements and models. The plan also describes where parameters of interest may be extrapolated from existing data.

This chapter is structured as an analysis plan might be structured for a specific risk assessment on land-applied biosolids. Following the introduction, management needs, including parameters requiring estimation, and data quality objectives are discussed. Then the plan for the characterization of exposure, including the selection of

measures of exposure, the detection of microbes, the issue of background levels of pathogens and the estimation of fate, transport, uptake, and dosage are discussed. The plan for the characterization of effects follows, including the selection of measures of effect, establishing cause and effect and dose-response models for infection. Methods for predicting disease, including the existence of infection thresholds and the role of immunity and epidemiological methods are also discussed. Finally, the plan for risk characterization is set forth, including the issue of standards, the possibility of tiered analysis, the weight-of-evidence approach, probabilistic assessment and uncertainty analysis.

The emphasis in this chapter is on aspects of analysis plans that are unique to risk assessments for biosolids-derived pathogens rather than risk assessments for pathogens in general. Therefore, some dose-response and epidemiological information is deemphasized. However, many opportunities for research, observational studies, and methods development are identified to improve and provide more defensible risk assessments. Finally, because this is a generic framework for an analysis plan, it does not contain the level of detail that would be expected in an analysis plan for a specific site or a particular regulatory action. This report does not provide site-specific advice on how to prioritize data needs, models, or assessment endpoints.

5.2. MANAGEMENT NEEDS

Risk managers have two fundamental requirements of risk assessors. The assessment process must estimate risks to endpoints that are important to the decision, and the results must have sufficient quality to be reliable. That is, risk managers should be able to understand the reliability of the results and determine if that reliability supports the decision to be made.

5.2.1. Assessment Endpoints

In any risk assessment, the assessment endpoint is an explicit expression of the value that should be protected. In health assessments, the endpoint is a property of human health. National level risk assessments for pathogens in biosolids would likely be conducted by EPA's Office of Water, and therefore, risk managers from this office

would determine the appropriate assessment endpoints. These may include population-level endpoints or individual-level endpoints. For example, it may be desirable to estimate the probability of infection (individual endpoint), number of infections during a period of time (population endpoint), number of infections during an outbreak (population endpoint), disease incidence (population endpoint), or related endpoints. The endpoint may be cumulative (estimating risk from pathogens of all sources) or may focus on only those infections or illnesses that are estimated to result from pathogens in biosolids. The risk manager may also specify levels of infection or disease that are acceptable or that require regulatory action. If applicable, these levels, as well as other properties of the assessment endpoint, should be described in the analysis plan. A purpose of the analysis plan is to set forth methods for estimating the assessment endpoint. The assessment endpoints will allow EPA to determine the level of public health and environmental protection from pathogens in biosolids afforded by 40 CFR 503,determine protective buffer distances, or evaluate current operational standards and management practices.

5.2.2. Data and Data Quality

EPA (1998) recommends that risk assessors consider several general questions related to the selection of data for the assessment:

- How relevant will the results be to the assessment endpoint(s) and conceptual model(s)?
- Are there sufficient data of high quality to conduct the analyses with confidence?
- How will the analyses help establish cause-and-effect relationships?
- How will results be presented to address managers' questions?
- Where are uncertainties likely to become a problem?

The analysis plan also specifies data quality objectives for the risk assessment. The Superfund program provides a good model for specifying the type of information that is needed to ensure data quality, specifying necessary and optimal levels of data

quality, and identifying the means of obtaining this information from risk managers (U.S. EPA, 1994). These steps are described in Text Box 1.

In addition, in compliance with the *Information Quality Act* (Public Law 106-554), EPA published *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of information disseminated by the Environmental Protection Agency* (U.S. EPA, 2002b). EPA's Information Quality Guidelines build on ongoing efforts to improve the quality of the data and analyses that support EPA's various policy and regulatory decisions and programs.

Text Box 1.

Recommended Steps for Specifying Data Quality Objectives (modified from U.S. EPA, 1994).

1. State the Problem. Clearly specify the question that relates to pathogens in biosolids. Is the concern a generic national problem? Or is it a site-specific one? Has an infection or disease been observed where the cause is unknown? Or is the risk manager concerned with future prediction?
2. Identify the Decision. Identify the decision that must be made to solve the problem. For example, are new regulations required to prevent unacceptable risk to human health?
3. Identify Inputs to the Decision. Identify the information needed to make the decision and measurements, simulations, and other analyses that must be undertaken to provide that information. These are the major components of the analysis plan.
4. Define the Assessment Boundaries. Specify the conditions to be assessed, including the spatial area, the time period and the exposure scenarios to which the decision will apply and for which inputs must be generated.
5. Develop Decision Rules. Define conditions under which an action, such as the promulgation of new regulations, will be taken.
6. Specify Acceptable Limits of Decision Error. Define error rates that are acceptable to the risk manager.
7. Optimize the Design. Design a study in which new data are collected and design the use of existing data in exposure or effects models, such that the expected variance in parameters results in an acceptable limit in decision error.

5.3. PLAN FOR CHARACTERIZATION OF EXPOSURE

5.3.1. Measures of Exposure

The first step to planning the characterization of exposure is selecting the measures of exposure. In a human health risk assessment, these are measurable characteristics of pathogens that are used to quantify exposure to humans or contact with particular organ systems. Measures of exposure include several variables such as: (1) concentrations of particular pathogens in environmental media or components of these media (biosolids, biosolids-amended soil, air, water, clay, aerosols), (2) duration of each exposure (daily versus per event), and (3) number and frequency of discrete

exposures over a specified time period.[7] Measures of exposure to microbial pathogens may also include inputs to models of fate, transport, or exposure (e.g., doses to humans), as described below.

5.3.2. Detection of Pathogens

Following the selection of measures of exposure, the detection of pathogens is the first type of analysis considered in the analysis plan. As stated in the literature review (see the Appendix), one of the major data gaps related to pathogens in biosolids is a recent national survey regarding levels of particular pathogens in sewage sludge and biosolids. Appropriate sampling and analytical methods are also needed for detecting and quantifying particular pathogens in sewage sludge (biosolids). In developing standard measurement methods for pathogens in biosolids, careful evaluation should be placed on method precision, accuracy, representativeness, sensitivity, and reproducibility of measurement methods. This information is needed to support national-scale human health risk assessments of biosolids. In site-specific risk assessments, it is possible to analyze the biosolids, amended soil, water, air or bioaerosol of concern to estimate pathogen levels, though these methods have high levels of uncertainty. The only feasible current option for national scale risk assessments is to conduct an analysis of pathogens in biosolids at several application sites that are thought to be representative of such sites across the country. EPA randomly samples at many biosolids sites (74 between 2006−2007) in many states (35 between 2006−2007). 145 analytes are tested and reported in the Targeted National Sewage Sludge Survey (U.S. EPA, 2009b). However, no microbial agents are included in the Targeted National Sewage Sludge Survey.

5.3.2.1. *Bacteria*

Smith et al. (2005b, Chapter 4) describe detection and enumeration capabilities for bacterial pathogens that involve general or selective enrichment combined with

[7] Chemical risk assessments often define exposure units as low level daily exposure over a 70-year life span. Microbial risk assessments often define exposure units as per event (e.g., per meal, per serving, per swimming event). The unit of exposure definition should be clear about whether daily exposure is compounded over a lifetime.

selective culturing or PCR and molecular identification techniques. However, these experts acknowledge that the use of these methods to detect all potential pathogens in a sample might be too costly or require too much effort to be practical. Thus, the use of indicator organisms is recommended if adequate indicators and appropriate analytical methodology are available (Smith et al., 2005b, Chapter 4) (also see the section on the *Use of Indicator Species* below). Recent research on species-specific biosensors may also produce useful products for detecting pathogens in biosolids (e.g., Guntupalli et al., 2007).

Organic matter and high bacterial counts reduce recovery fraction for pathogens in biosolids or amended soils (Rusin et al., 2003b). The analysis plan should indicate the recovery rates for the detection technologies that will be used. For example, recovery percentages of bacterial pathogens in aerosols that are reported in the literature are currently about 10% (Lubick, 2007). Rusin et al. (2003a) had a recovery efficiency of 8.7% for *Staphylococcus aureus* in Class B biosolids. EPA has new standardized analytical methods for fecal coliforms and *Salmonella* (Federal Register 57 14219).

5.3.2.2. *Viruses*

Sampling and detection of viruses that are present at high levels in biosolids is much easier than demonstrating conclusively that viral agents are not present (NRC, 2002). The primary determinant of the ease of detection of viruses is whether they can be cell-cultured. All of the viral pathogens listed in the hazard characterization chapter can be cell-cultured (NRC, 2002; Straub et al., 2007). Methods used to recover viruses from sewage sludge have been optimized for the enteroviruses rather than for other enteric viruses (Goyal et al., 1984; Gerba and Smith, 2005). Therefore, risk assessors need to be aware that there is high uncertainty regarding concentrations of nonenteroviruses in raw sewage sludge and treated biosolids (Smith et al., 2005b, Chapter 8). In addition to the interlaboratory variabilities on methodology, other disadvantages of cell culture methods include the high cost, long time required for positive results (up to one month) and the presence of potentially toxic organic compounds and inorganic elements in sewage sludge.

PCR is an alternative family of methods for identifying viruses. These analyses are quick, relatively inexpensive and sensitive. Direct reverse transcriptase PCR (RT-PCR) detects nucleic acid sequences from active and inactive viral particles, and thus may overestimate exposure (Straub et al., 1994). Integrated cell-culture PCR (ICC-PCR) amplifies viruses in cell culture and amplifies viral RNA through enzymatic PCR. The NRC (2002) thought that ICC-PCR would be the method of choice because of the potential for cell culture alone to underestimate human exposure and for RT-PCR to overestimate exposure (Reynolds et al., 1996; NRC, 2002).

5.3.2.3. *Helminths*

Various assays for helminth eggs in biosolids are available, but no standard assay exists, mainly because quality-assurance and quality-control studies have not been published for many study protocols (NRC, 2002). Candidate methods are referenced in NRC (2002), each with different recovery percentages for *Ascaris* eggs. Many do not adequately consider sample preservation and pretreatment. Some of these are not very accurate. The Tulane assay is discussed with recovery percentages, but this assay may not be valid for detecting helminths such as *Trichuris trichiura* that have eggs of different densities from *Ascaris* (NRC, 2002).

5.3.2.4. *Protozoa*

Methods for detecting helminths may be applicable to protozoa if final sieve size of the collection filters is adjusted to the smaller size of *Giardia* and *Cryptosporidium*. Viability and infectivity assays for protozoa that are available for the analysis plan include vital dye staining, animal infectivity, cell culture or PCR. Recovery rates from biosolids are low; 10% recovery rate using the sedimentation technique and less than 3% using the flotation technique. On average 3.2−16.3% *Cryptosporidium* oocysts and 2.4−41.7% *Giardia* cysts are recovered (NRC, 2002).

5.3.3. Use of Indicator Species

Because of the wide range of pathogens found in human feces, domestic wastewater and biosolids, direct monitoring and quantification of all of the pathogens in

biosolids may not be practical for a site-specific risk assessment (Nappier et al., 2006). Indicator species are abundant and are typically nonpathogenic microorganisms that may be used to indicate the presence of a suite of pathogens. For example, fecal coliform density and *Salmonella* are used as indicators of wastewater treatment efficiency (40 CFR 136). Tests for indicator microorganisms should be relatively simple and routine (NRC, 2002). However, most indicators have been chosen to indicate treatment effectiveness rather than measures of pathogens that are quantitative and are more closely related to public health (Smith et al., 2005b, Chapter 4). Suggestions for criteria for selecting indicator organisms in water matrices are presented in Text Box 2.

Tanner et al. (2005) cite research in their laboratory and other literature to show that (a) there is approximately one human pathogenic bacterium per 1000 coliform bacteria in biosolids and (b) one human enteric virus in Class B biosolids per 1000 coliphage.

Bacteria and helminths. Indicators of a range of pathogens in biosolids are needed. It may not be feasible for individual risk assessors to develop these indicators in the analysis plans for individual risk assessments. Given the resistance of spore-forming bacteria to desiccation, indicators of these bacterial pathogens would need to behave similarly. The NRC (2002) discusses *Clostridium perfringens* as a potential indicator of the efficiency of disinfection. In particular, they

Text Box 2.

Criteria for Selecting an Ideal Indicator Organism in Water Matrices (adapted from Gerba [2009] and NRC [2004]).

Biological Attributes

- The organism should be useful for all types of water.
- The organism should be present whenever enteric pathogens are present.
- The organism should be present in greater numbers than pathogens (relates to ease of detection).
- The organisms should have a similar or longer survival time than the hardiest enteric pathogen.
- The organisms should have similar or greater transports to pathogens.
- The organism should not grow in water.
- The density of the indicator organism should have some direct relationship to the degree of fecal pollution and correlate with health risk.
- The organism should be a member of the intestinal microflora of warm-blooded animals.

Methods Attributes

- The testing method should be easy to perform with timely results.
- Method should be specific to desired target organism
- Method should have broad applicability in different water types.
- Method should have adequate precision.
- Method should have adequate sensitivity.
- Method should provide quantifiable results.
- Method may measure viability or infectivity.

provide references suggesting that its spores might be a surrogate for eggs of *Ascaris* because of its resistance to similar chemical and physical disinfection agents. Furthermore, Dowd et al. (1997) recommend thermotolerant clostridia as indicators of fecal contamination in bioaerosols. Pillai et al. (1996) found that clostridia and H_2S producers were detected on glass impingers at locations near biosolids-amended sites where traditional bacterial indicators (fecal coliforms and fecal streptococci) were not. Thus *Clostridium perfringens* may be a useful surrogate for a range of pathogens in the analysis plan. Risk assessors may consider indicators of anaerobic pathogens, but genera such as *Bifidobacterium* and *Bacterioides* cannot be reliably detected and therefore cannot be routinely monitored (NRC, 2002).

Viruses. Smith et al. (2005b, Chapter 5) summarize the suitability of selected agents as indicators of treatment performance and post-treatment risk for viruses. Only the latter is relevant here and is presented in Table 7. Bacteriophages are the only potential indicator viruses mentioned in NRC (2002) because of their presence in sewage. Somatic coliphage infects strains of *E. coli* and can be detected using simple, inexpensive methods (NRC, 2002). Lime is also included as a potential indicator of post-treatment risk for survived viruses in Smith et al. (2005b), presumably because enteric viruses should be eliminated with extended alkaline treatment. At this time, these indicators are qualitative. Risk assessors would need to do substantial testing to quantify relationships between these indicators and pathogens of potential concern. Risk assessors may consider the use of a model organism for the development of a risk assessment. For an example of how model organisms can be used in risk assessments, see Soller (2006).

5.3.4. Background Levels of Pathogens

The analysis plan should assess background levels of pathogens through measurement or extrapolation from regional values if available. Background levels of pathogens are levels in environmental media (soil, water, or air) not amended with or contaminated by biosolids. Background levels are due to colonization of media at the regional scale. For example, endospore-forming bacteria such as *Clostridium perfringens* are very common in soil. The risk assessment is only concerned with the

TABLE 7

Suitability of Select Agents as Indicators of Post-Treatment Risk for Viruses in Biosolids (Modified from Smith et al., 2005b)

Agent	Suitability
Adenoviruses	?
Ascaris	Yes
Coliphages	Yes
Clostridium perfringens spores	Yes
Enterococci	No
Enteroviruses	Yes
E. coli	No
Fecal coliforms	No

incremental risk from pathogens in biosolids or the cumulative risk from pathogens in biosolids-amended soil, rather than the risk from pathogens in soil alone.

Background levels of pathogens (and/or pathogenic factors) may be confounding contributors to risk. For example, in a study of aerosolized endotoxin concentrations downwind from a biosolids-amended site, Brooks et al. (2006, 2007b) found that levels of endotoxin and diversity of bacteria in aerosolized soil were sometimes above those associated with biosolids amended-soil, calling into question whether biosolids were the primary source of the endotoxin.

5.3.5. Environmental Fate of Pathogens

The survival or regrowth of pathogens should be estimated if the risk assessment is prospective (i.e., concerned with forecasting), and environmental media cannot be sampled at the time of interest. Regulations that limit contact with biosolids do not prevent environmental processes in the conceptual model such as aerosolization or erosion (see Figure 2) and the death or multiplication of pathogens (see Figure 3). Therefore, the analysis plan may include a plan for estimating pathogen fate. Most studies of the fate of pathogens in sewage sludge are concerned with predicting the reduction or inactivation of pathogens by treatment processes (e.g., Epstein, 2006; Gantzer, 2001). Straub et al. (1993) reviewed available studies of survival of pathogens in soil and sewage sludge and Pepper et al. (2008a) examined detection of viable pathogens in biosolids; both are pertinent to this analysis plan discussion. Gerba and Smith (2005) provide survival times of pathogens on soil and plants (see Table 8). Microorganism regrowth is also a possibility (Zaleski et al., 2005a,b).

Risk assessors should not use survival data from enteric organisms such as *E. coli* and *Salmonella* to estimate the much longer survival rates of bacterial pathogens that form spores or are encapsulated (such as *Mycobacterium* spp.). Instead, a spore forming organism like *Clostridium perfringens* may be a more appropriate indicator (Karpowicz et al., 2009) because it is typically found throughout a wide range of environmental conditions, including extreme temperatures, relative humidity, and UV levels (Brooks et al., 2004a).

TABLE 8

Survival Times of Pathogens in Soil and on Plants
Modified from Gerba and Smith (2005)

Pathogen	Soil		Plants	
	Highest Maximum	Typical Maximum	Highest Maximum	Typical Maximum
Bacteria	1 year	2 months	6 months	1 month
Viruses	6 month	3 months	2 months	1 month
Protozoa	10 days	2 days	5 days	2 days
Helminths	7 years	2 years	5 months	1 month

5.3.6. Transport of Pathogens

The conceptual model in Figure 2 describes several transport processes, including wind erosion, surface runoff and water erosion, aerial dispersal of bioaerosols, deposition on crops, leaching to groundwater and vector transport. Applying biosolids can affect the rate at which soil erodes (Moffet et al., 2005). The analysis plan needs to provide a plan for answering the questions of how far and in what concentrations pathogens will travel. Models are available for most transport processes, though they may have some limitations as described below.

5.3.6.1. *Water Erosion*

Water erosion is typically modeled using the universal soil loss equation or its modifications. Average annual soil erosion is the product of a rainfall erosivity index, soil erodibility factor, topographic factor, cropping factor and conservation practice factor (Wischmeier and Smith, 1978). The soil erodibility factor estimates the cohesive nature of a soil type and resistance to transport from raindrop impact and surface flow. While this factor is available for various soil types, to our knowledge, it has not been measured for biosolids or biosolids-amended soils. The crop management factor is specific to agricultural systems and can include tillage but could be adapted to forest, greenway, mineland, or other biosolids application sites. Significant soil disturbance resulting from tracked vehicles could be incorporated in the soil erodibility or crop management factors. A limitation is that this equation is not applicable to a specific storm or year. If erosion is expected to be a significant transport process, these analyses would need to be part of the analysis plan.

5.3.6.2. *Surface Runoff and Aqueous Transport*

Methods for estimating surface runoff should be described separately from erosion models in the analysis plan. For example, Montemagno et al. (2004) describe a modeling strategy for estimating surface water contamination by pathogens from agricultural sources, using the specific example of oocysts of *Cryptosporidium*. Both surface runoff and water erosion are simulated.

For site-specific assessments, it may be desirable to use a spatially explicit model to simulate transport from land to streams and through a watershed to recreational areas or water intakes. Better Assessment Science Integrating Point and Nonpoint Sources (http://www.epa.gov/waterscience/basins/) provides an integrated system for such assessments. Alternatively, simple models of dilution and transport in a generic stream can be used.

5.3.6.3. *Wind Erosion*

Wind erosion should be considered in areas where wind speeds are often above the 19.3 km/h required to initiate soil movement (Brady, 1974). Wind erosion of soils and landscapes is governed by 11 primary variables: soil erodibility, knoll erodibility, surface crust stability, soil ridge roughness, wind velocity, surface soil moisture, distance across field, sheltered distance, quantity of vegetative cover, kind of vegetative cover and orientation of vegetative cover (Woodruff and Siddoway, 1965). The Wind Erosion Equation, developed by Woodruff and Siddoway (1965) groups many of these variables into an erodibility factor (which increases with percentage of soil particles greater than 0.84 mm diameter), a ridge roughness factor, a climatic factor, a field length factor and a vegetative cover factor. Clearly, the erodibility factor could be specific to biosolids, but the climatic factor, which incorporates soil moisture, would also be affected by biosolids added to the surface of soil or incorporated in soil. Again, this equation is not applicable to a specific year or wind event. Also, the Wind Erosion Equation provides a measure of dislodged soil; the equation provides no estimates of the travel distance of the soil (Batie, 1983).

5.3.6.4. *Aerial Transport of Bioaerosols*

To estimate bioaerosol transport, a risk assessor should understand the mechanism of transport, the release rates of the different microbes, the dispersion of the bioaerosols, and the deposition of the microorganisms (Baertsch et al., 2007; Pillai, 2007). These quantities depend on whether pathogens are aerosolized during particular types of biosolids application or following application. Pathogens in bioaerosols and their transport may be measured or modeled. The analysis plan may

include measurement of pathogens in air as a source term for a dispersion model or near the human receptors of interest.

The sampling of bioaerosols involves the removal and concentration of biological particles from the air (Pillai and Ricke, 2002). Sampling bioaerosols poses a particular challenge, compared to sampling of biosolids. Impaction, impingement, gravity settling, filtration and electrostatic precipitation are options for concentrating microorganisms from bioaerosols, but efficiencies of collection can be low or uncertain (NRC, 2002; Pillai and Ricke, 2002). Where molecular assays are feasible, collection methods do not have to preserve the viability of microbes for identification. It should be noted that culture methods require viability. If molecular methods are used, a description of the targets that are detected should be discussed in the context of how they compare to culture methods (Pillai and Ricke, 2002). Although there is a standard method for assessing occupational exposures to bioaerosols in indoor environments, no comparable standard exists for outdoor environments (NRC, 2002). Due to insufficient testing of available methods, no recommendation has been established for a particular sampling method for bacteria in bioaerosols. Risk assessments are more robust if the authors describe methods and acknowledge caveats for testing sampling efficiencies of their equipment in the analysis plan. Risk assessors should also be aware that during transport, deposition, and sampling, bacteria as well as other pathogens can be desiccated or inactivated, resulting in failure to culture and an underestimation of the number of viable cells. The analysis plan should specify how sampled pathogens will be handled.

Furthermore, determining an appropriate spatial distribution of samples is a challenge for sampling bioaerosols. If tens of acres are amended with biosolids, substantial micrometeorological differences may result from differing topography, vegetation, and mechanical agitation (NRC, 2002). Wind direction and speed may vary during the sampling time. The orifices of bioaerosol samplers downwind may collect too small of a volume of air to obtain detectable levels of bacteria, even if they are present in bioaerosols. In addition, efficiency of samplers decreases with increased wind speed. Thus, appropriate statistical analysis (Spicer and Gangloff, 2000) and appropriate

numbers of replicates are uncertain. These issues should be addressed in the analysis plan.

Models are available to estimate transport of pathogens in bioaerosols (Dowd et al., 2000; Brooks et al., 2004b, 2005a; Eisenberg et al., 2006). "Point-source" transport models are appropriate for localized sources of biosolids, such as a storage pile, and "area-source" models are more appropriate for predicting concentrations of pathogens downwind from a large biosolids-amended field in which including the length and width of the field more accurately estimates aerosol loading rates (Dowd et al., 2000). Dowd et al. (2000) modified a standard point-source transport model to incorporate the expected reduction in microbial concentration with increased distance from the source. Variables included the inactivation rate of the microorganism, mean wind speed, diffusion constants, downwind distance from source and height of sample. Typically, the risk assessor needs to back-calculate the rates of release of microorganisms from the source using sampling data, because measurement is extremely difficult (Dowd et al., 2000). A mathematical error in Dowd et al. (2000) was corrected in Brooks et al. (2004b).

An empirical model is another option for estimating aerosolized pathogen concentrations with distance from the source. Brooks et al. (2005a) derived a linear regression model that estimated coliphage concentrations at various distances from the spray application location, normalized for initial microbial concentration and wind speed. The researchers conducted field tests with coliphage MS-2 added to water and sprayed with a biosolids spray application truck. Temperature was also observed to influence aerosol concentration (Brooks et al., 2005a). The relationship these researchers derived may not be applicable to other biosolids, application methods or regions, but the development of similar empirical models may be an objective of the analysis plan.

Correlations have been developed between microbial levels in biosolids and their concentrations emitted during disking (Paez-Rubio et al., 2006) and spreading with a slinger side-spreader (Paez-Rubio et al., 2007). These types of reconstructions permit risk assessors to avoid difficulties of detecting pathogens in aerosols.

Indicator species may be used to estimate transport of related pathogens. For example, the ratio between the concentration of indicator virus in aerosols and the

concentration in biosolids was used to estimate a value for airborne enteric virus (Coxsackievirus) in Dowd et al. (2000).

Even allowing for sampling limitations and recovery efficiency issues, measurement is probably superior to models (which are validated using measurements in any case). Many of the physicochemical interactions between pathogens and biosolids and between pathogens and other components of bioaerosols are difficult to model. For example, viruses have been observed to sorb strongly to biosolids particles but to aerosolize more easily if present in the liquid fraction of biosolids (Brooks et al., 2004a). The transport of large dust particles is not usually modeled. Moreover, during application, the aerosol plume at each location is detectable for only a short period of time (e.g., less than one minute per pass of a spray applicator in Tanner et al. [2005]). Potentially complicating factors include variation in terrain, topography, vegetation, micrometeorological conditions, biosolid composition, and biosolids land application processes. Also, the bioaerosol transport reconstruction in Paez-Rubio et al. (2006) tended to result in a lower concentration than what was measured. Thus, risk assessors should justify selection of particular models in the analysis plan.

5.3.7. Contact with Crops

Biosolids and associated pathogens can deposit to crop leaves following erosion, aerial transport, or rain splash. Pathogen residues on root and leaf crops can be measured or the deposition processes can be modeled. Because of the uncertainty of modeling, direct measurement of pathogens on select crops would be preferred. If measurement is not possible, risk assessors can estimate the biosolids residues on root and leaf crops based on standard crop exposure assumptions (U.S. EPA, 1997), though these assumptions do not account for aerosolized pathogens depositing directly on leaves. Gale (2005b) offers assumptions that 10% of root crops were consumed unwashed or that 90% of soil was removed by washing prior to consumption.

Gale (2005a,b) describes ramifications of using the arithmetic mean root crop concentration as an input to dose-response models. This statistic often overestimates the number of people who are exposed to pathogens, because where pathogens are spatially clustered, many individuals are not exposed. Thus, the analysis plan should

indicate that the arithmetic mean exposure concentration (if used) may give a conservative estimate of the number of people exposed.

5.3.8. Uptake and Dosage

The analysis plan should include methods for estimating inhalation, ingestion, and dermal exposure when consideration of those routes of exposure is appropriate (see conceptual model discussion). For example, the dose of aerosolized pathogens to a person during a period of time may be estimated by measuring or modeling concentrations of microbes at a specific distance from the source and the inhalation rate over a period of time.

5.3.9. Exposure Factors

EPA does not have standard exposure factors for use in risk assessments of pathogens in biosolids. However, many of the exposure factors and assumptions described in EPA's *Exposure Factors Handbook* (U.S. EPA, 1997) and *Child-specific Exposure Factors Handbook* (U.S. EPA, 2008), which was designed for use in human exposure assessments for chemical contaminants, are pertinent. These include general exposure factors (e.g., drinking water intake rates, soil ingestion rates including for the pica child scenario, inhalation rates, body weight, body surface area), food ingestion factors (e.g., fruit and vegetable intake rates and water contents) and activity factors (e.g., time spent outdoors). This and other risk assessment guidance is available from the Risk Assessment Information System (U.S. DOE, 2006).

Some of the exposure factors in EPA (1997) may not be pertinent to risk assessments for pathogens in biosolids. For example, activity factors that estimate time spent outdoors may not be as relevant for a risk assessment of bioaerosols generated during biosolids application as the duration of the application process. The percentage of inhaled particles that would be ingested should be specific to biosolids-generated aerosols. Pepper et al. (2006) describe studies that use a factor of 10%, and Brooks et al. (2005b) uses 50%. Haas et al. (1999) recommend exposure factors that are relevant to risk assessments for pathogens. While many of these factors are analogous

to those in EPA (1997), others are more pertinent to risk assessments for pathogens (e.g., proportion of pathogens that are transferred to and from hands).

5.4. PLAN FOR CHARACTERIZATION OF EFFECTS

5.4.1. Measures of Effect

A measure of effect is a measurable quantity that is used to estimate the effects of exposure (to biosolids-derived pathogens) on the assessment endpoint. In this problem formulation, assessment endpoints include aspects of human health estimated at the individual level or population level. The analysis plan describes the measures of effect for the risk assessment. Suter et al. (2000) summarized considerations in selecting measures of effect for ecological risk assessments of chemical contaminants. These considerations were adapted here for pathogens in biosolids and at least the first two considerations are necessary to meet the definition of a measure of effect:

- Corresponds to an assessment endpoint (usually infection or illness)
- Endpoint is quantifiable or binary (e.g., infected or not infected)
- Makes use of existing data
- Is readily measured/detected (e.g., illness endpoint can be diagnosed, likelihood of disease given infection has been characterized, infection can be directly measured)
- Is of appropriate temporal and spatial scale (symptoms that occur within a timeframe where causality is relatively easier to establish versus chronic sequelae that occur far removed in time from infection)
- Is appropriate to the exposure route (pathogen can infect the tissues that are relevant for the exposure route)
- Is diagnostic of particular pathogens (pathogens can be grouped based on symptom sets)
- Shows low variability, increasing the likelihood of detecting an effect (Variability in response of the population is not a problem if it is well characterized, e.g., rate of movement between susceptible, infected, and resistant status is known. Variability in pathogen infectivity and virulence can introduce significant uncertainty into estimates. Particularly if strain/serovar/isolate variations are known to be highly variable.)

- Is broadly applicable to different locations (For national level microbial risk assessment information on prevalence of strains with different properties is important. Local immunity may also increase variability and therefore uncertainty.)
- Is a standard test or measurement method

Measures of effect are derived from laboratory studies (e.g., rat or mouse ingestion or bioaerosol inhalation studies) or epidemiological studies designed around biosolids application or disease outbreaks (controlled human clinical studies involving ingestion or inhalation are likely rare or nonexistent). Studies of disease outbreaks are often used to validate measures derived from animal models. The most applicable data would come from studies with biosolids, but other studies of pathogens can provide relevant data, especially in the absence of studies of biosolids. Additional health endpoints such as psychological effects may be considered in the problem formulation stage of a specific risk assessment.

Measures of effect in this problem formulation for biosolids-derived pathogens may include probability of infection (individual measure), number of infections during a period of time (population measure), number of infections during an outbreak (population measure), disease incidence (population measure) or related measures.

5.4.2. Establishing Cause and Effect

As noted later in the literature review (see the Appendix), a causal association between exposures to pathogens in biosolids and adverse effects on human health has not been documented based on principles of epidemiology. However, some experts point out this may be “too high a bar” to achieve before demonstrating more genuine concerns about potential effects. Risk assessors should examine relevant data both supporting and refuting a cause-and-effect relationship. This is most important in locations where biosolids are being implicated for observed disease symptoms.

Principles for establishing causality are described in Hill (1965). These include strength of association, consistency of association (e.g., observation of the symptoms near multiple biosolids application sites), specificity of association, relationships between timing of application and onset of symptoms, biological gradient

(dose-response relationship), plausibility of the causative relationship, coherence of evidence, observation in experiments and analogy to known associations (e.g., occupational exposures to pathogens in biosolids). Hill's principles may be used to determine whether land application of biosolids may cause particular diseases. The analysis plan for site-specific risk assessments where disease has been observed might include methods that are not pertinent to national-scale assessments. For example, deoxyribonucleic acid (DNA) fingerprinting methods can be used to determine whether pathogens isolated from sick individuals have originated from land-applied biosolids (Dowd and Pillai, 1999; NRC, 2002). Santo Domingo et al. (2007) provide methods to track sources of fecal pollution. Although there are no EPA approved methods, there are standardized DNA fingerprinting methods used in the PulseNet labs that monitor foodborne pathogens.[8] Epidemiological studies are discussed below. Risk assessors for site-specific human health assessments might also benefit from reviewing EPA's guidance for identifying ecological stressors to specific aquatic ecosystems in the *Stressor Identification Guidance Document* (U.S. EPA, 2000) and the supporting Causal Analysis/Diagnosis Decision Information System Web site (http://www.epa.gov/caddis/) to see how approaches used there might apply to pathogens in land-applied biosolids.

5.4.3. Dose-Response Models for Infection

Empirical effects models quantify the relationship between the dose of a microbial agent and frequency of a particular adverse outcome, such as infection, disease, or mortality. These models may assume a minimum infective dose greater than one organism (which for microbial pathogens is supported by little evidence, see below) or a no-threshold continuous dose-response function. These empirical models allow risk assessors to estimate risk at low doses of pathogens. The equations are derived from exposure of humans or animal models to various concentrations of pathogens. For a more detailed treatment of dose-response models, see the *Draft Protocol for Microbial Risk Assessment to Support Human Health Protection for Water-Based Media* (U.S. EPA, 2009a).

[8] The standard lab protocols are published on the Centers for Disease Control and Protection website: http://www.cdc.gov/pulsenet/protocols.htm (accessed 6/30/10).

Microbial dose-response models mathematically represent the measure of the dose that yields the probability of a given adverse effect. For microbes, the models are required to be biologically plausible and should consider effects due to microbial strain variations (Coleman et al., 2004) and that a population of humans exposed to infectious microbes will receive a distribution of actual doses (Haas et al., 1999).

Several dose-response models have been used to assess human health risk from microbial agents. These models include exponential dose-response, beta-Poisson dose-response and simple and variable threshold models. These models have been used to assess risk from waterborne and foodborne exposures to microbial agents and recently in risk assessments of pathogens in dewatered, land-applied biosolids (Dowd et al., 2000; Brooks et al., 2004b, 2005b; Eisenberg et al., 2004). Table 9 provides examples of dose-response models for microbial agents that may be associated with biosolids. All of these examples, except for endotoxins, pertain to the endpoint of infection rather than disease. Only the endotoxins use a threshold model which is consistent with their chemical nature; doses of all of the other microbial agents were as whole organisms. Further reading and examples of critically analyzed dose-response curves for microbial agents that may be associated with biosolids are presented in Chapter 9 of *Quantitative Microbial Risk Assessment* (Haas et al., 1999) and in studies by Coleman and Marks (2000), Teunis et al. (2005), and EPA (2009a).

Infective doses reported for various bacteria, viruses, and protozoan and helminth parasites are tabulated in Epstein (2006) and Gutierrez (2005). However, Haas et al. (1999) argue that most evidence supports the independent action (or single-organism) hypothesis that even a single organism can initiate an infection in a susceptible host. Risk assessors might view reported infective doses as doses where infection becomes likely rather than actual thresholds.

Dose-response models represent major information gaps for risk assessments related to pathogens in biosolids (Coleman and Marks, 1998). Most dose-response models have been developed from human or animal feeding studies or from investigations of outbreaks caused by contaminated food without apparent biosolids involvement (Haas et al., 1999; Teunis et al., 2008b). Dose-response relationships are not available for all of the pathogens potentially found in biosolids (see hazard

TABLE 9

Examples of Dose-Response Models for Microbial Agents
(adapted from U.S. EPA, 2009a)

Organism	Measure of Exposure	Model	Endpoint	Reference
Adenovirus 4	Dose	Exponential	Human infection	Crabtree et al. (1997), Haas et al. (1999)
Campylobacter jejuni	Dose	Beta-Poisson	Human infection	Haas et al. (1999), Medema and Smeets (2004), Teunis et al. (1996)
Campylobacter jejuni	Dose	Hypergeometric beta-Poisson	Infection Illness: conditional on infection	Teunis et al. (2005)
Coxsackievirus B3	Dose	Exponential	Human infection	Dowd et al. (2000), Brooks et al. (2004b), Haas et al. (1999)
Cryptosporidium parvum	Dose	Exponential	Human infection	Dupont et al. (1995), Haas et al. (1996, 1999), Okhuysen et al. (1999), EPA (2006b)
Cryptosporidium parvum	Dose	Beta-Poisson	Human infection	Englehardt and Swartout (2004)
Cryptosporidium parvum	Dose	Beta-Poisson	Gastroenteric illness	Englehardt and Swartout (2006)
Echovirus 12	Dose	Exponential	Human infection	Haas et al. (1999)
Echovirus 12	Dose	Beta-Poisson	Human infection	Teunis et al. (1996), Regli et al. (1991), Rose and Sobsey, (1993), Rose and Gerba (1991)
Endamoeba coli	Dose	Beta-Poisson	Human infection	Haas et al. (1999)

TABLE 9 cont.

Organism	Measure of Exposure	Model	Endpoint	Reference
E. coli (0111)	Dose	Beta-Poisson	Human infection	Ferguson and June (1953), Haas et al. (1999)
E. coli (055)	Dose	Beta-Poisson	Human infection	June et al. (1953), Haas et al. (1999)
E. coli O157:H7	Dose	Beta-Poisson	Human infection	(Teunis et al., 2008b)
E. coli O157:H7	Dose	Hypergeometric beta-Poisson	Human infection	(Teunis et al., 2004)
Endotoxin	Concen-tration in air	Threshold	Decreased lung efficiency, Organic Toxic Dust Syndrome	Baker et al. (1986)
Enteric virus	Dose	Beta-Poisson	Human infection	Gerba et al. (2002)
Giardia lamblia	Dose	Exponential	Human infection	Haas et al. (1999), Regli et al. (1991), Rose and Gerba (1991), Rose et al. (1991), Teunis et al. (1996)
Hepatitis A virus	Dose	Exponential	Human infection	Haas et al. (1999)
Legionella	Dose	Exponential	Human infection	Armstrong and Haas (2008)
Norovirus	Dose	Hypergeometric function; one-hit	Human infection	Teunis et al. (2008a)
Poliovirus I	Dose	Beta-Poisson	Human infection	Regli et al. (1991), Rose and Sobsey (1993), Rose and Gerba (1991)
Poliovirus I	Dose	Exponential	Human infection	Haas et al. (1999), Regli et al. (1991)

TABLE 9 cont.

Organism	Measure of Exposure	Model	Endpoint	Reference
Poliovirus III	Dose	Beta-Poisson	Human infection	Rose and Sobsey (1993), Regli et al. (1991), Rose and Gerba (1991)
Rotavirus	Dose	Exponential Beta-Poisson Log-probit	Human infection	Ward et al. (1986), Haas et al. (1999), Gerba et al. (1996b) Regli et al. (1991), Rose and Gerba (1991), Rose and Sobsey (1993)
Rotavirus	Dose	Hypergeometric bet-Poisson	Human infection	Teunis and Havelaar (2000)
Salmonella spp.	Dose	Beta-Poisson	Human infection	Rose and Gerba (1991)
Salmonella spp.	Dose	Gompertz log	Human infection	Coleman and Marks (2000), Coleman et al. (2004), Soller et al. (2007)
Salmonella spp.	Dose	Generalized linear mixed models and fractional polynomials of dose	Human infection	Bollaerts et al. (2008)
Salmonella serovar Anatum	Dose	Beta-Poisson	Human infection	McCullough and Eisele (1951), Haas et al. (1999)
Salmonella serovar Typhi	Dose	Beta-Poisson	Human infection	Dowd et al. (2000), Brooks et al. (2004b), Haas et al. (1999), Rose and Gerba (1991)
Salmonella serovar Typhi	Dose	Fractional polynomials	Human infection	Namata et al. (2008)

TABLE 9 cont.				
Organism	Measure of Exposure	Model	Endpoint	Reference
Shigella	Dose	Beta-Poisson	Human infection	Haas et al. (1999)
Vibrio cholera	Dose	Beta-Poisson	Human infection	Haas et al. (1999)

characterization chapter). Dose-response relationships are also not available for inhaled microorganisms (NRC, 2002). As stated in the literature review (see the Appendix), the percentage of inhaled pathogens that are ingested is unknown. Dose-response models are also not available for dermal exposure. Furthermore, few dose-response models are available for disease.

5.4.4. Predicting Disease

Existing risk assessment studies for pathogens in biosolids estimate risk of human infection rather than risk of disease (see literature review in the Appendix). If limited by existing data, risk assessments for diseases caused by pathogens in biosolids would be highly uncertain.

Disease is a function of a "triad," the interaction of pathogen, host, and environment. All three factors figure into assessing the incidence of disease in individuals, and the problem formulation should include a plan for the analysis of all three aspects. The pathogen is the causative agent of the disease. Whereas chemicals are generally assumed to elicit human-comparable responses in appropriate animal models, pathogens are more host-specific. Pathogens can elicit adverse responses either through their own biological activity within the host or through the production of toxic byproducts.

The second aspect of disease is the host condition. The disease manifestation can vary considerably among infected individuals based on nutritional and health status, and immune profile. Individuals in good health with a history of prior exposure to similar strains of pathogens are less likely to exhibit pronounced symptoms than individuals in poor health or without prior exposure. Host susceptibility can be considered on an individual level or a population level (Balbus et al., 2000). Immunity is one of the most important parameters influencing the risk from pathogens in biosolids, based on the Eisenberg et al. (2004) model. The analysis plan should specify whether groups of individuals of particular immune status are assessment endpoint entities in the risk assessment. Protocols to incorporate immune status or other pathogen susceptibility factors (pregnancy, age) into risk assessments have not been robustly developed (NRC, 2002).

The environment aspect of the triad refers to conditions which promote or retard the ability of the organism to survive in various media and which contribute or limit the spread of the organisms to a receptor. For the most part, the environment is addressed in the exposure components of the conceptual model and is pertinent to infection rather than disease. An assessment of disease incidence cannot proceed without an understanding of these factors and how they influence individual components of the model.

5.4.4.1. *Risk Assessment Model*

Colford et al. (2003) and Eisenberg et al. (2004, 2005, 2006) developed methodologies to assess risks to human health from pathogens in biosolids and biosolids-amended soil. While many of the processes in the model are those described in this chapter (fate, transport, uptake, exposure pathways to groundwater and aerosols), not all aspects of those models may be needed depending on the particular risk assessment. Eisenberg et al. (2008) argue that raw sludge data on pathogen levels combined with fate and transport modeling for treatment effects is a helpful tool. Eisenberg et al. (2004, 2005, 2006) also modeled secondary transmission, which is important for estimating the total burden of disease. However, secondary transmission of pathogens is not unique to pathogens found in biosolids and, as such, is not discussed further here. This document is concerned first and foremost about any potential risks of primary infection because secondary transmission is not a concern unless a primary infection has occurred. If secondary transmission is suspected, other sources of information may be investigated on how to address that. For a few resources regarding secondary transmission refer to Section 3.

5.4.4.2. *Role of Epidemiology*

Epidemiological assessments of land-applied biosolids would provide much needed information concerning the potential for adverse impact to human health following land application of biosolids. Presently, few data exist to provide insight as to whether a causative association exists between applied biosolids and adverse health effects. Temporal and spatial relationships between the time of application and onset of

symptoms or other indicators would identify key routes of exposure to assess the validity of the conceptual models presented here and to prioritize different exposure scenarios. Epidemiological assessments would focus on studies or disease reports (clustering of illness cases) that can draw a link between those individuals living in close proximity to sites of application and members of farm families and workers who apply biosolids to determine if those individuals have a higher incidence of disease over time.

Risk assessments which use epidemiological studies of sites on or near places of biosolids application would be based on the collection of several key data sets. First, the data should indicate whether individuals living on or near lands receiving biosolids have a higher incidence of infection compared with cohorts at more distant locations. Second, data should identify temporal relationships between time and duration of application and onset of symptoms. Such relationships could indicate potential route of exposure—rapid onset may suggest aerosol exposure, whereas delayed disease may indicate an alternate exposure route. Third, data should establish a concordance of symptoms which could also help to determine the route of exposure and whether a single or multiple pathogens are responsible for the effects. Collectively, this information would help to determine if there is a significant microbial risk associated with the use of Class B biosolids and, if so, to help to refine conceptual models and to identify the primary data and methods needed for the risk assessment.

Additionally, epidemiological information for biosolids amendments should focus on plausible exposure scenarios and the characterization of potentially exposed cohorts. First, identifying the exposure settings provides a link between biosolids application and environmental transport of pathogens and exposure points for human contact. Second, data on potentially exposed populations should be identified using information on proximity to the site of biosolids application, climatic conditions, and temporal relationships between posited exposures and the onset of infection or clinical symptoms. The selection of appropriate cohorts is important along with the availability of supporting medical information, such as isolates of pathogens and/or serology demonstrating infection within a time frame that corresponds with a plausible exposure scenario (e.g., time of application, environmental transport, exposure point, exposure route, infection, etc.).

Risk assessors should be aware of the difficulties in conducting an epidemiological study of biosolids exposure. For example, various symptoms may be associated with one pathogen, and various pathogens can cause similar symptoms (Simmonds, 2005). In addition, low level health effects are very difficult to detect with epidemiological studies because very large population sizes would need to be studied to observe effects. However, preliminary work has been done to scope epidemiological designs to assess acute health effects and community-level exposure to treated sewage sludge (Class B biosolids) in North Carolina and Virginia (Heaney et al., 2006).

5.5. PLAN FOR RISK CHARACTERIZATION

The analysis plan should include a plan for conducting the risk characterization, which is the phase of risk assessment that integrates the characterization of exposure and the exposure-response relationships to estimate the likelihood of health effects endpoints. Care should be taken that the units between the exposure parameters and the dose-response parameters agree or the uncertainty introduced by differences in units can be qualitatively or quantitatively characterized. For example, exposure age groups may encompass different age groups than dose-response data. If “healthy adults” is defined as age 18−55 with specific “health” indicators determined by doctor’s examination (e.g., in a clinical trial) for the dose-response parameter and the exposure parameter population group is defined as “adults age 16−60” then the nuances of those differing groupings and the possible effects those differences have on the risk characterization should be discussed.

5.5.1. Screening Risk Assessment

The analysis plan describes whether the risk assessment will include a screening-level risk characterization to eliminate pathways, pathogens, or scenarios that are clearly not of concern. A screening analysis typically makes use of effects standards or benchmarks, but no acceptable level of pathogens in biosolids has been established. Screening analysis can also eliminate pathways using qualitative information (e.g., obvious lack of contact between pathogens and residents in an area devoid of residences). A risk assessor with sufficient resources could develop critical

distances for potential risk associated with the bioaerosol transport pathway, and thus eliminate scenarios where there are no people within the critical distance. Screening analysis is usually conducted for information-rich risk assessment topics, which is not the expected situation for risk assessments for pathogens in biosolids.

5.5.2. Weight of Evidence

If multiple lines of evidence are expected, the analysis plan should explain how these results would be weighed. Each line of evidence links an exposure estimate with an effects estimate, and qualitative or quantitative weights may be given to the combined risk estimate. Evidence from measures of pathogen levels in aerosols might be weighted more than evidence from modeled estimates based on measures of biosolids-amended soils. Evidence from well-designed epidemiological studies might be weighted more than evidence from rodent studies that have not been corroborated with epidemiological evidence. Suter et al. (2000) provide the following criteria for weighing evidence: relevance to the assessment endpoint, demonstrated relationship between exposure and response, temporal scope of evidence compared to temporal variance, spatial scope of evidence compared to spatial area of interest, data quality, number of observations, and uncertainty of evidence. Given the paucity of exposure and effects data for risk assessments of land-applied biosolids, weight-of-evidence procedures may not be possible or infrequent.

5.5.3. Uncertainty Analysis

Uncertainty analysis is the component of the risk characterization that reveals the uncertainties of the exposure or risk estimate in quantitative or qualitative terms. The management goal of uncertainty analysis may be simply to describe uncertainties, to rank uncertainties, or to calculate a probabilistic endpoint. In the case of pathogens in biosolids, probabilistic endpoints might be generated from variability and uncertainty in measurements of pathogens in biosolids, outputs of transport models, or outputs of dose-response models. Haas et al. (1999) divided uncertainty into parameter uncertainty, which is related to measurement, and model uncertainty, which is related to the structure of the equations (e.g., whether an important factor was missing from the

model or not). The uncertainties associated with the sampling and modeling methods are described above in the relevant sections. When new data are needed and cannot be obtained, risk pathways that cannot be assessed are a source of uncertainty and should be described in the analysis plan. Risk assessors need to distinguish between pathways that are unquantifiable and pathways that are deemed negligible based on evidence. For further information in uncertainty analysis see EPA (1997, 2009a).

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Evanylo, G.K. 1999. Agricultural land application of biosolids in Virginia: Managing biosolids for agricultural use. Virginia Cooperative Extension, Virginia Tech, Blacksburg, VA. (Evanylo, 1999, 625223)
This reference summarizes pertinent information on agricultural use of biosolids application. Information includes agronomic rates, application methods, and storage.

Ferguson, W.W. and R.C. June. 1952. Experiments on feeding adult volunteers with *Escherichia coli* 111 B_4: A coliform organism associated with infant diarrhea. Am. J. Hyg. 55(2):155−169. (Ferguson and June, 1952, 625224)
Dose-response information for *E. coli*.

Gale, P. 2003. Using event trees to quantify pathogen levels on root crops from land application of treated sewage sludge. J. Appl. Microbiol. 94:35−47. (Gale, 2003, 624974)
This reference documents a modeling approach to predict the incremental exposure of root crops, at point of harvest to enteric pathogens from sewage sludge applied to agricultural land according to current regulations and guidance (Safe Sludge Matrix). Land application of sewage sludge treated by conventional processes (achieving 2-log removal) is predicted to increase the exposures of root crops to *Salmonella* and *Cryptosporidium* by counts of 0·070 and 0·033 kg^{-1}, respectively.

Gale, P. 2005a. Pathogens in biosolids—microbiological risk assessment. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure. Compost Science and Utilization, J.E. Smith, Jr., P. Millner, W. Jakubowski, and N. Goldstein, Ed. The J.G. Press, Inc., Emmaus, PA. p. 177−194. (Gale, 2005, 625225)
This reference is a book chapter that examines microbiological risk assessment of biosolids derived pathogens.

Gale, P. 2005b. Land application of treated sewage sludge: Quantifying pathogen risks from consumption of crops. J. Appl. Microbiol. 98(2):380–396. (Gale, 2005, 624976)
This reference assesses risks to human health from the consumption of vegetable crops grown on agricultural land to which treated sewage sludge has been properly applied.

Gale, P. and G. Stanfield. 2001. Towards a quantitative risk assessment for BSE in sewage sludge. J. Appl. Microbiol. 91(3):365−369. (Gale and Stanfield, 2001, 624973)
This reference documents a quantitative risk assessment to determine the risk of BSE transmission to humans and cattle through the application of sewage sludge to agricultural land. The study finds that risks to humans through consumption of vegetable crops are acceptably low. Risks to cattle are higher because of higher exposure to soil and greater susceptibility. The model demonstrates that sewage sludge alone cannot sustain the BSE epidemic in the UK cattle herd.

Gantzer, C.P., P. Gaspard, L. Galvez, A. Huyard, N. Dumouthier and J. Schwartzbrod. 2001. Monitoring of bacterial and parasitological contamination during various treatment of sludge. Water Res. 35(16):3763−3770. (Gantzer et al., 2001, 104901)
Impact of various sludge treatments on pathogens (*Salmonella* and nematode eggs) and indicators (*E. coli*, *Enterococci*, sulfite-reducing anaerobes spores). Various treatment processes were analyzed: 4 biological, 3 chemical, 1 heat, 2 storage and 4 sampling campaigns were carried out.

Gattie, D.K. and T.J. McLaughlin. 2004. A high-level disinfection standard for land-applied sewage sludges (biosolids). Environ. Health Perspect. 112(2):126−131. (Gattie and McLaughlin, 2004, 635170)
Complaints associated with land-applied sewage sludges primarily involve irritation of the skin, mucous membranes, and the respiratory tract accompanied by opportunistic infections.

Gavett, S.H., and H.S. Koren. 2001. The role of particulate matter in exacerbation of atopic asthma. Int. Arch. Allergy Immunol. 124(1−3):109−112. (Gavett and Koren, 2001, 016168)
Results of these studies suggest that transition metals in ambient particulate matter promote the formation of reactive oxygen species and subsequent lung injury, inflammation, and airway hyperresponsiveness leading to airflow limitation and symptoms of asthma.

George, C.L., H. Jin, C.L. Wohlford-Lenane, et al. 2001. Endotoxin responsiveness and subchronic grain dust-induced airway disease. Am. J. of Physiol. Lung C. 280(2):L203−L213. (George et al., 2001, 104906)
Findings demonstrate that subchronic inhalation of grain dust extract results in the development of chronic airway disease only in mice sensitive to endotoxin but not in mice that are genetically hyporesponsive to endotoxin, suggesting that endotoxin is important in the development of chronic airway disease.

Gerba, C.P. 2005. Enteric viruses in biosolids. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure. Compost Science and Utilization, J.E. Smith, Jr., P. Millner, W. Jakubowski, and N. Goldstein, Ed. The J.G. Press, Inc., Emmaus, PA. (Gerba, 2005, 625226)
This reference characterizes enteric viruses found in biosolids.

Gerba, C.P. 2009. Environmental indicators. In: Environmental Microbiology, 2nd ed., R.M. Maier, I.L. Pepper, and C.P. Gerba, Ed. Academic Press, NY. p .485−498. (Gerba, 2009, 635174)
A reference book including a discussion about environmental indicators.

Gerba, C.P. and V. Smith, Jr. 2005. Sources of pathogenic microorganisms and their fate during land application of wastes. J. Environ. Qual. 34:42−48. (Gerba and Smith, 2005, 624982)
This reference is a review that identifies: (1) the types of wastes applied to land in which pathogens may be present, (2) current concerns with the risks of these practices, and (3) future research needs.

Gerba, C.P., J.B. Rose and C.N. Haas. 1996a. Sensitive populations: Who is at greatest risk? Intl. J. Food Microbiol. 30(1−2):113−123. (Gerba et al., 1996, 080225)
This reference is a review that identifies groups of individuals who would be at the greatest risk of serious illness and mortality from water and foodborne enteric microorganisms. Groups include the very young, the elderly, pregnant women, and the immunocompromised.

Gerba, C.P., J.B. Rose, C.N. Haas and K.D Crabtree. 1996b. Waterborne rotavirus: A risk assessment. Water Res. 30(12):2929−2940. (Gerba et al., 1996, 635177)
Dose-response information for Rotavirus.

Gerba, C.P., I.L. Pepper and L.F. Whitehead, III. 2002. A risk assessment of emerging pathogens of concern in the land application of biosolids. Water Sci. Technol. 46(10):225-230. (Gerba et al., 2002, 624979)
This reference is a risk analysis that assesses the most likely emerging pathogens to survive treatments for Class B biosolids before land application. The study concludes that Adenoviruses and Hepatitis A virus are the most thermally resistant viruses that can survive prolonged periods of time in the environment. And injection of liquid biosolids into the soil results in significant reduction of the concentration of the viruses. This reference also provides dose-response information for enteric viruses.

Germolec, D.R., R.S H. Yang, M.F. Ackerman et al. 1991. Toxicology studies of chemical mixtures of 25 groundwater contaminants: Immune suppression in B6C3F mice. Fund. Appl. Toxicol. 13(3):377−387. (Germolec et al., 1991, 635179)
Mixture of 25 common groundwater contaminants frequently found near toxic waste dumps, as determined by EPA surveys, tested on mice. These results suggest that longterm exposure to contaminated groundwater may represent a risk to the immune system in humans.

Gibbs R.A., C.J. Hu, G.E. Ho and L. Unkovich. 1997. Re-growth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. Water Sci. Technol. 35(11−12):269−275. (Gibbs et al., 1997, 635180)
Soil amended with biosolids could not be considered free from pathogens for at least 1 year following amendment.

Goyal, S.M., S.A. Schaub, F.M. Wellings et al. 1984. Round robin investigation of methods for recovering human enteric viruses from sludge. Appl. Environ. Microbiol. 48(3):531−538. (Goyal et al., 1984, 624983)
This reference documents the selection of a tentative standard method for detection of viruses in sludge.

Graczyk, T.K., R. Knight, R.H. Gilman and M.R. Cranfield. 2001. The role of non-biting flies in the epidemiology of human infectious diseases. Microbes Infect. 3(3):231−235. (Graczyk et al., 2001, 635182)

This reference is a review on the role of nonbiting flies in the epidemiology of human infections and diseases. Topics include the feeding and reproductive habits, subceptible populations, and transmission in hospital environments.

Gregersen, P., K. Grunnet, S.A. Uldum, B.H. Andersen and H. Madsen. 1999. Pontiac fever at a sewage treatment plant in the food industry. Scand. J. Work Environ. Health 26(3):291−295. (Gregersen et al., 1999, 624984)

This reference documents an investigation of workers contracting Pontiac fever after repairing a decanter for sludge concentration at a sewage treatment plant. It was concluded that the fever was caused by *L. pneumophila* emitted to the environment by the uncovered decanter.

Guntupalli, R., R.S. Lakshmanan, J. Hu et al. 2007. Rapid and sensitive magnetoelastic biosensors for the detection of *Salmonella typhimurium* in a mixed microbial population. J. Microbiol. Meth. 70(1):112−118. (Guntupalli et al., 2007, 624986)

This reference reports the employable performance of a wireless, magnetoelastic biosensor designed to selectively detect *Salmonella typhimurium* in a mixed microbial population.

Gutierrez, S. 2005. Why a workshop on emerging infectious disease agents and issues associated with animal manures, biosolids, and other similar by-products? In: Compost Science and Utilization, J.E. Smith, Jr., P. Millner, W. Jakubowski, and N. Goldstein, Ed. The J.G. Press, Inc., Emmaus, PA. p. 25−31. (Gutierrez, 2005, 625227)

This reference provides tabulated information on pathogen infectious doses.

Haas, C.N., C.S. Crockett, J.B. Rose, C.P. Gerba and A.M. Fazil. 1996. Assessing the risks posed by oocysts in drinking water. J. Am. Water Works Assoc. 88(9):131−136. (Haas et al., 1996, 635186)

Dose-response information for *Cryptosporidium parvum*.

Haas, C.N., J.B. Rose and C.P. Gerba. 1999. Quantitative Microbial Risk Assessment. John Wiley and Sons, Inc., New York, NY. (Haas et al., 1999, 625228)

This book is a commonly cited source of dose-response relationships and other tools and methods for microbial risk assessment.

Heaney, C., W. Steven and A. Lowman. 2006. Study design features of a longitudinal investigation of acute health effects and community-level Class B biosolids exposure: Subject recruitment and exposure assessment. Epidemiology. 17(6) (Suppl. S): S443−S443. Available at http://journals.lww.com/epidem/Fulltext/2006/11001/Study_Design_Features_of_a_Longitudinal.1189.aspx. (Heaney et al., 2006, 625233)

This reference presents strengths and weaknesses of two study design features of a proposed investigation of acute health effects and community-level exposure to treated sewage sludge (Class B biosolids) in North Carolina (NC) and Virginia (VA).

Heffernan, J.M., R.J. Smith and L.M. Wahl. 2005. Perspectives on the basic reproductive ratio. J. R. Soc. Interface 2(4):281–293. (Heffernan et al., 2005, 635189)
This reference is a review of the basic reproductive ratio, R0. The review includes a survey of recent use of R0 in assessing emerging diseases, such as severe acute respiratory syndrome and avian influenza, a number of recent livestock diseases, and vector-borne diseases malaria, dengue and West Nile virus.

Herr, C.E.W., A. zur Nieden, M. Jankofsky, N.I. Stilianakis, R.H. Boedeker and T.F. Eikmann. 2003. Effects of bioaerosol polluted outdoor air on airways of residents: A cross section study. Occup. Environ. Med. 60:336−342. (Herr et al., 2003, 104909)
Residents exposed to bioaerosol pollution were shown to report irritative respiratory complaints similar to mucous membrane irritation independently of perceived odors.

Hethcote, H.W. 2000. The mathematics of infectious diseases. SIAM Rev. Ind. Appl. Math 42(4):599−653. (Hethcote, 2000, 635191)
Risk assessment methods.

Hill, A.B. 1965. The environment and disease: Association or causation? Proc. R. Soc. Med. 58:295−300. (Hill, 1965, 071664)
A classic article summarizing several aspects to consider in interpreting causation.

Hinckley, G.T., C.J. Johnson, K.H. Jacobson et al. 2008. Persistence of pathogenic prion protein during simulated wastewater treatment. Environ. Sci. Technol. 42(14):5254−5259. (Hinckley et al., 2008, 104896)
Results suggest that if prions were to enter municipal wastewater treatment systems, most would partition to activated sludge solids, survive mesophilic anaerobic digestion, and be present in treated biosolids.

Horswell, J., J. Hewitt, J. Prosser et al. 2010. Mobility and survival of *Salmonella typhimurium* and human adenovirus from spiked sewage sludge applied to soil columns. J. Appl. Microbiol. 108(1):104−114. (Horswell et al., 2010, 635194)
This reference documents that the presence of sewage sludge can significantly influence the transport and survival of bacterial pathogens in soils, probably because of the presence of organic matter. Environmental contamination by virus is unlikely because of strong soil adsorption.

ILSI (International Life Sciences Institute). 2000. Revised Framework for Microbial Risk Assessment. ILSI Risk Science Institute, Washington, DC. (ILSI, 2000, 625240)
This reference provides a comprehensive framework for use in microbial risk assessments.

Jin, Y., J.T. Sims and K. Kniel. 2008. Effect of Land Application of Wastes on the Fate and Transport of Pathogens in Soil. U.S. Department of Agriculture, National Research Initiatives (NRI). Available at http://www.reeis.usda.gov/web/crisprojectpages/207060.html. (Jin et al., 2008, 635196)

Objectives: (1) determine the survival potential of selected viruses in representative manures and biosolids, and on plants grown in soils amended with these materials; (2) measure sorption and desorption of viruses by manures, biosolids, and soils; (3) examine the soil factors and manure/biosolids application methods controlling the leaching potential of viruses using large undisturbed soil columns containing benchmark soil series of the Mid-Atlantic U.S.; and (4) elucidate the mechanisms of virus retention and transport during leaching under saturated and unsaturated flow conditions in controlled laboratory column studies.

John, D.E. and J.B. Rose. 2005. Review of factors affecting microbial survival in groundwater. Environ. Sci. Technol. 39(19):7345–7356. (John and Rose, 2005, 635197)

This reference is a review that quantitatively examines a number of published studies that evaluated survival and inactivation of public-health-related microorganisms in groundwater.

June, R.C., W.W. Ferguson and M.T. Worfel. 1953. Experiments in feeding adult volunteers with *Escherichia coli* 55 B_5: A coliform organism associated with infant diarrhea. Am. J. Hyg. 57:222−236. (June et al., 1953, 624987)

Dose-response information for *E. coli.*

Karpowicz, E., A. Novinscak, F. Barlocher et al. 2009. qPCR quantification and genetic characterization of *Clostridium perfringens* populations in biosolids composted for 2 years. J. Appl. Microbiol. 108(2):571−581. (Karpowicz et al., 2009, 635199)

This reference reports a study to improve the understanding of *C. perfringens* persistence in composted biosolids by monitoring its presence and studying its genetic diversity. The study found that composting did not significantly decrease the number of *C. perfringens* cells. High genetic diversity of *C. perfringens* isolates present in composted biosolids is reported for the first time.

Khuder S., S.A. Milz, M. Bisesi, R. Vincent, W. McNulty and K. Czaikowski. 2007. Health survey of residents living near farm fields permitted to receive biosolids. Arch. Environ. Occup. Health. 62(1):5−11.(Khuder et al., 2007, 104914)

Survey findings suggest an increased risk for certain respiratory, gastrointestinal, and other diseases among residents living near farm fields on which the use of biosolids was permitted.

Lang, N.L. and S.R. Smith. 2007. Influence of soil type, moisture content and biosolids application on the fate of *Escherichia coli* in agricultural soil under controlled conditions. J. Appl. Microbiol. 103(6):2122−2131. (Lang and Smith, 2007, 635203)

Evaluates survival of *E.coli* of biosolid origin once land applied.

Lang, N.L., S.R. Smith, D.M. Bellett-Travers, E.B. Pike and C.L. Rowlands. 2003. Decay of *Escherichia coli* in soil following the application of biosolids to agricultural land. Water Environ. J. 17(1):23−28. (Lang et al., 2003, 598358)

This reference presents a field experiment on decay of *E. coli* in a sandy loam soil, amended with enhanced and conventionally treated biosolids following spring and autumn application of sewage sludge.

Lang, N.L., M.D. Bellett-Travers and S.R. Smith. 2007. Field investigations on the survival of *Escherichia coli* and presence of other enteric microorganisms in biosolids-amended agricultural soil. J. Appl. Microbiol. 103(5):1868−1882. (Lang et al., 2007, 104916)

Evaluates survival of *E. coli* of biosolid origin once land applied.

Lewis, D.L. and D.K. Gattie. 2002. Pathogen risks from applying sewage sludge to land. Environ. Sci. Technol. 36(13):287A−293A. (Lewis and Gattie, 2002, 598361)

This reference provides an overview for issues related to pathogen risks from applying sewage sludge to land.

Lewis, D.L. and D.K. Gattie. 2003. Comment on "Evidence for the absence of *Staphylococcus aureus* in land applied biosolids". Environ. Sci. Technol. 37(24):5836. (Lewis and Gattie, 2003, 635205)

Comments on Rusin et al. (2003a).

Lewis, D.L., S. Shepherd, D.K. Gattie, S. Sanchez and M. Novak. 2001. Enhanced susceptibility to infection from exposure to gases emitted by sewage sludge: A case study. In: Proceedings of the Water Environment Federation, Innovative Uses of Biosolids and Biosolids Management. Water Environment Federation, Alexandria, VA. p. 392−399. Available at http://www.ingentaconnect.com/content/wef/wefproc/2001/00002001/00000004/art00042. (Lewis et al., 2001, 104919)

This reference documents symptoms exhibited by residents living in a Greenland, NH neighborhood where Class B biosolids were applied in 1995. The residents experienced severe irritation of the eyes, skin, and mucous membrane followed by respiratory and gastrointestinal illnesses.

Lewis, D.L., D.K. Gattie, M.E. Novak, S. Sanchez and C. Pumphrey. 2002. Interactions of pathogens and irritant chemicals in land-applied sewage sludges (biosolids). BMC Pub. Health. 2:11. Available at http://www.biomedcentral.com/1471-2458/2/11. (Lewis et al., 2002, 598363)

This reference documents the reported symptoms of residents of biosolids land application sites and suggests that an increased risk of infection may occur when allergic and nonallergic reactions to endotoxins and other chemical components irritate skin and mucus membranes and thereby compromise normal barriers to infection.

Lubick, N. 2007. Estimating aerosolized contaminants from Class B biosolids. Environmental Science and Technology Online News. (accessed 4/4/07). (Lubick, 2007, 635209)

Lytle, D.A., E.W. Rice, C.H. Johnson and K.R. Fox. 1999. Electrophoretic mobilities of *Escherichia coli* O157:H7 and wild-type *Escherichia coli* strains. Appl. Environ. Microbiol. 65(7):3222−3225. (Lytle et al., 1999, 598366)

This reference documents the electrophoretic mobilities (EMs) of *E. coli* O157:H7 and wild-type strains. The EPMs of *E. coli* O157:H7 strains differed from those of wild-type strains. As the suspension pH decreased, the EPMs of both types of strains increased.

McCullough, N.B. and C.W. Eisele. 1951. Experimental human salmonellosis: I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray dried whole egg. J. Infec. Dis. 88:278−289.(McCullough and Eisele, 1951, 598367)

Dose-response information for *Salmonella*.

McFarland, M.J. 2000. Biosolids Engineering. McGraw Hill, New York, NY. (McFarland, 2000, 598369)

This is a general reference book covering broad but detailed aspects of biosolids too.

Mead, P.S., L. Slutsker, V. Dietz et al. 1999. Food related illness and death in the United States. Emerg. Infect. Dis. 5(5):607−625. (Mead et al., 1999, 635213)

This CDC reference is widely cited for foodborne disease rates.

Meckes, M. 2011. E-mail from M. Meckes, Office of Research and Development, National Risk Management Laboratory, Cincinnati, OH to M. Troyer, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH, January 20, 2011.

Research experience in this laboratory is that solids content in biosolids cake is frequently 15−25%.

Medema, G. and P. Smeets. 2004. The interaction between quantitative microbial risk assessment and risk management in the water safety plan. Kiwa Water Research/Delft University. (Medema and Smeets, 2004, 635214)

Dose-response information for *Campylobacter jejuni*.

Moffet, C.A., R.E. Zartman, D.B. Wester and R.E. Sosebee. 2005. Surface biosolids application: effects on infiltration, erosion, and soil organic carbon in Chihuahuan Desert grasslands and shrublands. J. Environ. Qual. 34(1):299–311. (Moffet et al., 2005, 635215)

This reference documents the measurement of infiltration and erosion of surface application of biosolids. Infiltration increased with increasing biosolids application rate. Soil erosion was reduced by the application of biosolids; however, the extent of reduction in erosion depended on the initial erodibility of the site. Surface application of biosolids has important hydrological consequences on runoff and soil erosion in desert grasslands that depend on the rate of biosolids applied, and the site and biosolids characteristics.

Monroe, S.S., T. Ando and R.I. Glass. 2000. Introduction: human enteric caliciviruses—an emerging pathogen whose time has come. J. Infect. Dis. 181(Suppl. 2):S249−S251. (Monroe et al., 2000, 635216)

This reference provides the background information on how caliciviruses became an important human pathogen of concern and lays out the current state of the field.

Montemagno, C.D., L.L. Yeghiazarian and P. Kalita. 2004. Field calibration and berification of a pathogen transport model. Water Environment Research Foundation, Alexandria, VA.. Available at http://www.werf.org/AM/Template.cfm?Section=Preparing_a_Report&Template=/CM/ContentDisplay.cfm&ContentFileID=22. (Montemagno et al., 2004, 635217)

This reference documents an integrated modeling strategy to quantify the risk of surface drinking water contaminated by waterborne pathogens, in particular the oocysts of *C. parvum*, from agricultural nonpoint pollution sources.

Namata, H., M. Aerts, C. Faes and P. Teunis. 2008. Model averaging in microbial risk assessment using fractional polynomials. Risk Analysis 28(4):891−905. (Namata et al., 2008, 635218)

Dose-response information for *Salmonella* (Tyhpi).

Nappier, S.P., M.D. Aitken and M.D. Sobsey. 2006. Male-specific coliphages as indicators of thermal inactivation of pathogens in biosolids. Appl. Environ. Microbiol. 72:2471−2475. (Nappier et al., 2006, 598373)

This reference documents the use of coliphase as indicators of thermal inactivation of pathogens in biosolids. The approach began by isolating coliphases from municipal wastewater sludge from biosolid samples after thermophilic anaerobic digestion to evaluate the susceptibility of specific groups to thermal inactivation. IF+ RAN phages were found to be the majority organism present in digested biosolids, likely reduced via a mechanism different from heat inactivation. Therefore, III F+ RNA coliphages should function as a potential indicator.

NRC (National Research Council) 1983. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC. (NRC, 1983, 194806)

This report is often referred to as the “Red Book” and is commonly cited as a risk assessment framework for chemicals.

NRC (National Research Council). 2002. Biosolids Applied to Land: Advancing Standards and Practices. National Academy Press, Washington, DC. (NRC, 2002, 598374)

This reference evaluates the standards and practices of biosolids land application. Recommendations were made to use improved risk assessment methods to establish standards for chemical and pathogen hazards; conduct a new national survey of chemicals and pathogens in sewage sludge; establish a framework for an approach to implement human health investigations; and to increase the resources devoted to EPA's biosolids program.

NRC (National Research Council). 2004. Indicators for Waterborne Pathogens. National Academies Press, Washington, DC. (NRC, 2004, 635221)

This report discusses water quality indicators, ecology of waterborne pathogens and indicators, attributes of indictors, and monitoring approaches.

NRC (National Research Council). 2009. Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, DC. (NRC, 2009, 180073)

This report expands on the 1983 NRC “Red Book” framework by developing planning and scoping and problem formulation as well as decision making frameworks for chemical risk assessment.

Nwachuku, N. and C.P. Gerba. 2004. Emerging waterborne pathogens: Can we kill them all? Curr. Opin. Biotechnol. 15(3):175−180. (Nwachuku and Gerba, 2004, 598375)

This reference is a review that focuses on the need to better understand why children are at greater risk from environmentally transmitted pathogens and the need for special consideration for this age group when developing environmental standards.

Okhuysen, P.C., C.L. Chappell, J.H. Crabb, C.R. Sterling and H.L. DuPont. 1999. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. J. Infect. Dis. 180(4):1275−1281. (Okhuysen et al., 1999, 635223)

Dose-response information for *Cryptosporidium parvum*.

Paez-Rubio, T. and J. Peccia. 2005. Estimating solar and nonsolar inactivation rates of airborne bacteria. J. Environ. Eng. 131(4):612−617. (Paez-Rubio and Peccia, 2005, 598376)

This reference documents the pilot-scale bioaerosol reactor experiments that independently measure the solar and nonsolar (absence of solar radiation) inactivation rates of airborne *Mycobacterium parafortuitum* and *E. coli*. *E. coli* was more susceptible to airborne decay than *M. parafortuitum* at all relative humidity (RH) levels tested. RH strongly influenced solar and nonsolar airborne inactivation rates in both bacteria. These inactivation rates for both bacteria were greatest at moderate RH levels.

Paez-Rubio, T., X. Hua, J. Anderson and J. Peccia. 2006. Particulate matter composition and emission rates from the disk incorporation of Class B biosolids into soil. Atmos. Environ. 40(36):7034−7045. (Paez-Rubio et al., 2006, 119151)

Field studies were conducted at Central Arizona biosolids land application sites to characterize the physical, chemical, and biological content of aerosols produced during biosolids disking and the content of bulk biosolids and soil from which the aerosols emanate. Source aerosol concentrations and calculated emission rates reveal that disking is a substantial source of biosolids-derived aerosols.

Paez-Rubio, T., A. Ramarui, J. Sommer, H. Xin, J. Anderson and J. Peccia. 2007. Emission rates and characterization of aerosols produced during the spreading of dewatered Class B biosolids. Environ. Sci. Technol. 41(10):3537−3544. (Paez-Rubio et al., 2007, 598702)

This reference documents the measurement of aerosol emission rates produced during the spreading of dewatered Class B biosolids onto agricultural land. Rates were determined in multiple independent experimental runs by characterizing both the source aerosol plume geometry and aerosol concentrations of PM10, total bacteria, heterotrophic plate count bacteria (HPC), two types of biosolids indicator bacteria, endotoxin, and airborne biosolids regulated metals. Based on the land application rates of spreaders used in this study, an estimated 7.6 ± 6.3 mg of biosolids were aerosolized for every 1 kg (dry weight) applied to land.

Peccia, J., H.M. Werth, S. Miller and M. Hernandez. 2001. Effects of relative humidity on the ultraviolet induced inactivation of airborne bacteria. Aerosol Sci. Technol. 35:728−740. (Peccia et al., 2001, 598841)

This reference assesses the response of aerosolized *Serratia marcescens*, *Bacillus subtilis*, and *Mycobacterium parafortuitum* to ultraviolet irradiation at different relative humidity (RH) levels in a 0.8 m3 completely-mixed chamber. When RH exceeded approximately 50%, sorption increased markedly and a sharp concurrent drop in UV-induced inactivation rate was observed.

Pepper, I.L., K.L. Josephson, R.L. Bailey, M.D. Burr and C.P. Gerba. 1993. Survival of indicator organisms in Sonoran Desert soil amended with sewage sludge. J. Environ. Sci. Heal. A 28(6):1287–1302. (Pepper et al., 1993, 635230)

This study documented the survival period of fecal coliforms after field land application and also regrowth after rainfall events.

Pepper, I.L., J.P. Brooks and C.P. Gerba. 2006. Pathogens in biosolids. Adv. Agron. 90:1−41. (Pepper et al., 2006, 599024)

This reference is a book chapter review of the human pathogens as potential hazards associated with biosolids and its land application.

Pepper, I.L., H. Zerzghi, J.P. Brooks and C.P. Gerba. 2008a. Sustainability of land application of Class B biosolids. J. Environ. Qual. 37:58−67. (Pepper et al., 2008, 635231)

This reference documents the sustainability of long term land application of biosolids. Viable pathogens were not detected in soil 6 months after the application of biosolids, even after 20 annual applications.

Pepper, I.L., J.P. Brooks and C.P. Gerba. 2008b. Pathogens and indicator organisms in Class B biosolids before and after the Part 503 Sludge Rule. In: Proceeds of the Water Environment Federation's Technical Exhibition and Conference WEFTEC 2008: Session 51 through Session 60. Water Environment Federation, Alexandria, VA. p. 3955−3961. (Pepper et al., 2008, 635228)

Indicator loads are similar to loads prior to the promulgation of the Part 503 Rule, whereas pathogen loads have decreased.

Pepper, I.L., J.P. Brooks, R.G. Sinclair, R.L. Gurian, C.P. Gerba. 2010. Pathogens and indicators in United States Class B biosolids: National and historic distributions. J. Environ. Qual. 39(November-December). E-pub doi:10.2134/jeq2010.0037. Available online http://www.virginiabiosolids.com/pdf/Pathogens%20in%20biosolids-2010.pdf. (Pepper et al., 2010, 697223)

Incidence of indicator organisms and pathogens in Class B biosolids from 18 wastewater treatment plants across the United States.

Pillai, S.D. 2007. Bioaerosols from land applied biosolids: Issues and needs. Water Environ. Res. 79(3):270−278. (Pillai, 2007, 624872)

This reference provides an overview for the issues and needs related to bioaerosols from land applied biosolids. The focus is on current information and technology gaps related to estimating public health risks.

Pillai, S.D. and S.C. Ricke. 2002. Bioaerosols from municipal and animal wastes: Background and contemporary issues. Can. J. Microbiol. 48:681−696. (Pillai and Ricke, 2002, 621261)

This reference is a review that synthesizes the information related to bioaerosols and addresses the contemporary issues associated with bioaerosols from municipal and animal wastes, with a focus on pathogens.

Pillai, S.D., K.W. Widmer, S.E. Dowd and S.C. Ricke. 1996. Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. Appl. Environ. Microbiol. 62(1):296−299. (Pillai et al., 1996, 615793)

This reference documents the occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. Indicators such as H2S producers and pathogenic clostridia were present in locations having significant physical agitation of the sludge material.

Regli, S., J.B. Rose, C.N. Haas and C.P. Gerba. 1991. Modeling the risk from Giardia and viruses in drinking-water. J. Am. Water Works Assoc. 83:76−84. (Regli et al., 1991, 635235)

Dose-response information for *Echovirus* and *Giardia lamblia*.

Reynolds, K.A., C.P. Gerba and I.L. Pepper. 1996. Detection of infectious enteroviruses using cell culture/PCR procedure. Appl. Environ. Microbiol. 62(4):1424−1427. (Reynolds et al., 1996, 635236)

This is a key reference for ICC-PCR. First research group to demonstrate the method's advantages.

Rose, J.B. and C.P. Gerba. 1991. Use of risk assessment for development of microbial standards. Water Sci. Technol. 24:29−34. (Rose and Gerba, 1991, 635237)

Dose-response information for *Echovirus*, *Giardia lamblia*, *Poliovirus*, and *Salmonella*.

Rose, J.B. and M.D. Sobsey. 1993. Quantitative risk assessment for viral contamination of shellfish and coastal waters. J. Food Protect. 56(12):1043−1050. (Rose and Sobsey, 1993, 635239)

Dose-response information for *Echovirus* and *Poliovirus*.

Rose. J.B., C.N. Haas and S. Regli. 1991. Risk assessment and control of waterborne giardiasis. Am. J. Pub. Health. 81(16):709−713. (Rose et al., 1991, 635238)

Dose-response information for *Giardia lamblia*.

Rusin, P.A., S.L. Maxwell, J.P. Brooks, C.P. Gerba and I.L. Pepper. 2003a. Evidence for the absence of *Staphylococcus aureus* in land applied biosolids. Environ. Sci. Technol. 37(37):4027−4030. (Rusin et al., 2003, 624873)

This reference documents an investigation to determine if *Staphylococcus aureus* is present in biosolids using samples from 15 biosolids land application sites across the U.S. The results suggest that biosolids are not a likely source of *S. aureus* human exposure or infection.

Rusin, P.A., S.L. Maxwell, J.P. Brooks, C.P. Gerba and I.L. Pepper. 2003b. Response to comment on "Evidence for the absence of *Staphylococcus aureus* in land applied biosolids". Environ. Sci. Technol. 37(24):5836. (Rusin et al., 2003, 624875)

This is a rebuttal to D.L. Lewis and D.K. Gattie (2003).

Sahlstrom L., B. de Johg and A. Aspan. 2006. *Salmonella* isolated in sewage sludge traced back to human cases of salmonellosis. Lett. Appl. Microbiol. 43(1):46−52. (Sahlstrom et al., 2006, 104921)

This study demonstrates that *Salmonella* spp. isolated in sewage treatment plants (STP) originate from infected humans and survive treatment at STP. It also highlights the risk of spreading resistant *Salmonella* strains from sewage sludge to the environment.

Santo Domingo, J.W., D.G. Bambie, T.A. Edge and S. Wuertz. 2007. Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution. Water Res. 41(16):3539−3552. (Santo Domingo et al., 2007, 624876)

This reference examines different viewpoints associated with the practical use of microbial source tracking (MST) to identify critical research gaps, proposes a priority-based timeline to address them, and outlines emerging technologies that will likely impact the future of source tracking.

Schiffman, S.S., J.M. Walker, P. Dalton et al. 2000. Potential health effects of odor from animal operations, wastewater treatment facilities and recycling byproducts. J. Agromed. 7(1):7−81. (Schiffman et al., 2000, 635244)

This report summarizes the conclusions from the Workshop regarding the potential mechanisms responsible for health symptoms from ambient odors.

Selvaratnam, S. and J.D. Kunberger. 2004. Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana creek adjacent to farmland amended with treated sludge. Can. J. Microbiol. 50(8):653−656. (Selvaratnam and Kunberger, 2004, 104926)

These results suggest that surface runoff from the farmland treated with sludge is strongly correlated with higher incidence of Amp(R) and fecal coliforms at one test site.

Shusterman, D. 1992. Critical review; The health significance of environmental odor pollution. Arch. Environ. Health. 47: 76−87. (Shusterman, 1992, 076187)

Noxious environmental odors may trigger symptoms by a variety of physiologic mechanisms, including exacerbation of underlying medical conditions, innate odor aversions, aversive conditioning phenomena, stress-induced illness, and possible pheromonal reactions.

Sidhu, J.P. and S.G. Toze. 2009. Human pathogens and their indicators in biosolids: A literature review. Environ. Int. 35(1):187−201. (Sidhu and Toze, 2009, 635247)

This reference is a review that summarizes reported literature on the numbers and fate of enteric pathogens and indicators in biosolids. The advantages and limitations of the use of conventional and alternative index and model microorganisms for the prediction of pathogen presence in biosolids are also discussed.

Simmonds, C. 2005. Pathogens in biosolids: Risks and regulations. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, Compost Science and Utilization, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Ed. The J.G. Press, Inc., Emmaus, PA. p. 231−238. (Simmonds, 2005, 624879)

This reference is a book chapter that focuses on the risks posed by pathogens in biosolids and the guidelines that have arisen in Australia and the U.S. to manage these risks.

Skanavis, C. and W.A. Yanko. 1994. Evaluation of composted sewage sludge based soil amendments for potential risks of salmonellosis. J. Environ. Health. 56(7):19. (Skanavis and Yanko, 1994, 104931)

The distribution of salmonellae serotypes did not suggest a strong correlation between the occurrence of *Salmonella* in the compost products and *Salmonella* infections in the community. Analysis of exposure demonstrated that the probability of infection was low in most scenarios.

Smieszek, T. 2009. A mechanistic model of infection: Why duration and intensity of contacts should be included in models of disease spread. Theor. Biol. Med. Model. 6:25. Available at http://www.tbiomed.com/content/6/1/25. (Smieszek, 2009, 635250)
This reference documents the difference in modeling outcomes between a mechanistic model and a constant per-contact transmission probability. In particular, cases with many different contacts (super-spreaders) have much lower expected numbers of secondary cases when using the mechanistic model.

Smith, J.E., P D. Millner and N. Goldstein. 2005a. Highlights, insights, and perspectives on infectious disease agents in sewage sludge and animal manure in the United States. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, Compost Science and Utilization, J.E. Smith, Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk, Ed. The J.G. Press, Inc., Emmaus, PA. p. 3−23. (Smith et al., 2005, 624882)
This reference is a book chapter that highlights the core principles and findings from the workshop in 2001 and to provide a historical, policy, and regulatory framework on issues related to infectious disease agents in sewage sludge and animal manure in the United States.

Smith, J.E., Jr., P. Millner, W. Jakubowski, N. Goldstein and R. Rynk. 2005b. Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, Compost Science and Utilization. The J.G. Press, Inc., Emmaus, PA. (Smith et al., 2005, 624883)
This reference provides a comprehensive evaluation of facts and critical gaps in knowledge about the growth, survival and dissemination of infectious agents from wastewater sludge and animal manures and the prospects for disinfection with a variety of existing treatment technologies.

Soller, J.A. 2006. Use of microbial risk assessment to inform the national estimate of acute gastrointestinal illness attributable to microbes in drinking water. J. Water Health. 4(Suppl 2):165−186. (Soller, 2006, 635253)
Use of model organism in microbial risk assessments. Risk assessment tool.

Soller, J.A. and J.N.S. Eisenberg. 2008. An evaluation of parsimony for microbial risk assessment models. Environmetrics 19(1)61−78. (Soller and Eisenberg, 2008, 635254)
Mathematical equations for dynamic risk assessment models.

Soller, J.A., J.N.S. Eisenberg, D.M. Eisenberg et al. 2006a. Research digest: use of risk assessment to evaluate human health risks associated with pathogens in biosolids. Water Environment Research Foundation, Alexandria. (Soller et al., 2006, 624885)
This research digest summarizes the findings of the results of the two phases of WERF project 98-REM-1. The risk assessment framework provides a mechanism to discuss biosolids management microbial risk using a common metric for comparison of treatment methods, management alternatives, and to set risk-based standards for microbial contaminants in biosolids.

Soller, J.A., A.W. Olivieri, J.N.S. Eisenberg, J. DeGeorge, R.C. Cooper and G. Tchobanoglous. 2006b. A public health evaluation of recreational water impairment. J Water Health. 4(1):1−19. (Soller et al., 2006, 104932)
Disease transmission model method.

Soller, J.A., E.Y. Seto and A.W. Olivieri. 2007. Application of microbial risk assessment techniques to estimate risk due to exposure to reclaimed waters. WateReuse Foundation, Final Project Report, WRF-04-011. (Soller et al., 2007, 635256)

Dose-response information for *Salmonella*.

Spicer, R.C. and J.J. Gangloff. 2000. Limitations in application of Spearman's rank correlation to bioaerosols sampling data. Am. Ind. Hyg. Assoc. J. 61(3):362−366. (Spicer and Gangloff, 2000, 624887)

This reference documents the model simulations comparing two zones of microbial data from the same environment. The simulations indicated that nonparametric statistical treatment of bioaerosol data as currently recommended for building assessment purposes has limitations. An inordinately high Type II error (failure to reject a null hypothesis which is actually not true) is especially apparent when there are small numbers of samples.

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This reference documents the comparative effects of humidity on the preharvest survival of microbial pathogens on cantaloupe, lettuce, and bell peppers. *C. perfringens* may be an acceptable indicator of bacterial contamination and survival in various environments and on different types of crops.

Straub, T.M., I.L. Pepper and C.P. Gerba. 1993. Hazards from pathogenic microorganisms in land-disposed sewage sludge. Rev. Environ. Contam. Toxicol. 132:55−91. (Straub et al., 1993, 624899)

This reference provides an overview of the characteristics of microbial pathogen hazards in sewage sludge.

Straub, T.M., I.L. Pepper, M. Abbaszadegan and C.P. Gerba. 1994. A method to detect enteroviruses in sewage sludge-amended soil using the PCR. Appl. Environ. Microbiol. 60(3):1014–1017. (Straub et al., 1994, 635260)

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This is a general reference book on ecological risk assessment.

Tanner, B.D. 2004. Aerosolization of microorganisms and risk of infection from reuse wastewater residuals. Ph.D. Dissertation, Graduate School of Medicine, The University of Arizona, Tucson, AZ. (Tanner, 2004, 624902)
This reference documents 3 experiments conducted to characterize the concentration of microorganisms in biosolids, the plume of aerosols created during land application of biosolids, and the occupational risk of infection due to pathogens aerosolized during land application of biosolids in the United States.

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This reference documents bioaerosol emission rates and plume characteristics of bioaerosols generated during land application of liquid Class B biosolids. A comparison of aerosolization rates of coliphages and total coliform bacteria between liquid biosolids land application and inoculated groundwater during land application. The results indicate that some property of biosolids reduces aerosolization of microorganisms relative to groundwater and aerosolization of coliphages and coliform bacteria after liquid biosolids have been applied to land does not occur at detectable levels.

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This paper gives additional information on bioaerosols generated during land application.

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Dose-response information for Rotavirus.

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Dose-response information for *Giardia lamblia*.

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Risk assessment tool.

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This is an online resource of risk assessment tools.

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Status report on land application of biosolids. Issues examined include: (1) EPA and state biosolids program staff, (2) delegation of the biosolids program to the states, (3) extent to which biosolids are land applied in seven states, (4) responding to and tracking health complaints, (5) risk assessment and pathogen testing concerns, (6) EPA's relationship with a professional association, and (7) public acceptance concerns.

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This document has quantitative information on exposures experienced by children.

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Yanko, W.A. 2005. Bacterial pathogens in biosolids—emerging issues. In: Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure, Compost Science and Utilization, J.E. Smith, Jr., P. Millner, W. Jakubowski, and N. Goldstein, Ed. The J.G. Press, Inc., Emmaus, PA. p. 35−49. (Yanko, 2005, 624975)

This is a book chapter summarizing emerging pathogens of concern.

Yates, M.V., and S. Yates. 2007. Assessing the Fate of Emerging Pathogens in Biosolids. WERF Report: Protecting human health.01-HHE-3. IWA Publishing, London, U.K. (Yates and Yates, 2007, 104935)

Research on the fate of emerging pathogens during biosolids treatment and subsequent application on land. Specific objectives were to: (1) assess the fate of several emerging pathogens (e.g., Adenoviruses, Hepatitis A virus, *E. coli* O157:H7, Listeria, and Cryptosporidium) during Class A and Class B biosolids treatment processes; (2) based on the results of Objective 1, assess the fate and transport of the most significant organisms in biosolids applied to soil columns; and (3) assess the potential for selected pathogens to survive and be transported at a field site.

Zaleski, K.J., K.L. Josephson, C.P. Gerba and I.L. Pepper. 2005a. Survival, growth, and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil, and land applied biosolids. J. Residuals Sci. Technol. 2:49−63. (Zaleski et al., 2005, 624978)

This reference evaluates the potential for conversion of Class B to Class A biosolids with respect to salmonellae and fecal coliforms during solar drying in concrete lined drying beds. The results suggest that the use of concrete-lined beds created a situation in which moisture added as rainfall accumulated in the beds, promoting the growth of fecal coliforms and salmonellae added from external sources.

Zaleski, K.J., K.L. Josephson, C.P. Gerba and I.L. Pepper. 2005b. Potential regrowth and recolonization of *Salmonellae* and indicators in biosolids and biosolid-amended soil. Appl. Environ. Microbiol. 71(7):3701−3708. (Zaleski et al., 2005, 624980)

This reference is a review on issues related to the survival and potential regrowth of pathogenic and indicator bacteria in biosolids, compost, soil, and land-applied biosolids.

Zerzghi, H., C.P. Gerba, J.P. Brooks, I.L. Pepper. 2009. Long-term effects of land application of Class B biosolids on the soil microbial populations, pathogens, and activity. J. Residuals Sci. Technol. 7:51−61. (Zerzghi et al., 2010, 635670)

This reference documents a 20-year study that showed no long-term adverse effects at 20 annual biosolids land application sites and concludes that land application of biosolids at this particular site was sustainable throughout the 20-yr period, with respect to soil microbial properties.

APPENDIX
LITERATURE REVIEW

This appendix presents a literature review that summarizes the available information on microbial risks to humans posed by land-applied biosolids. The review is organized in terms of summary points, research and data gaps, relevant aspects of the National Research Council (NRC, 2002) recommendations on biosolids, and data and information available for phases of risk assessments (e.g., fate, transport, uptake, infectivity, risk assessment, causal analysis). Although some studies of pathogens in manures may be relevant to biosolids (e.g., models of pathogen transport), investigations of these untreated materials are beyond the scope of this report. This literature review was completed prior to developing the other chapters in this report.

SUMMARY POINTS

- The range of pathogens that may be present in biosolids is well understood, but the current national distribution of these pathogens, the variation with type of sewage sludge treatment, and standard analytical methods for detecting and quantifying pathogens in biosolids are not well understood or developed.
- Many analytical methods for detecting and quantifying pathogens focus on detecting deoxyribonucleic acid sequences rather than viable cultures.
- The use of indicator organisms to represent pathogens of concern has the potential to introduce large uncertainties into estimates of exposure.
- The use of pathogen-specific dose-response relationships coupled with the uncertainties of indicator organism modeling (the above point) could compound the difficulty in conducting a meaningful and scientifically defensible risk assessment.
- Risk assessments of pathogens in biosolids have been performed, but the emphasis has been on the use of particular transport models to quantify risks from a few pathogens to individuals at a distance from particular biosolids application sites. Eisenberg et al. (2004, 2005, 2006, 2008) have developed some risk assessment tools that may be useful for national-scale or other broad risk assessment.

- Some conceptual models for human health risk assessments of pathogens in biosolids that include detailed source descriptions, transport pathways and routes of exposure have been developed (Colford et al., 2003).

- Epidemiological studies of biosolids application sites are generally lacking and are problematic to conduct.

- An epidemiologic causal association between exposures to biosolids and adverse effects on human health has not been documented.

- Although the Unites States Environmental Protection Agency (EPA) has standard exposure factors and effects levels relevant to chemicals, some standard exposure factors and effects levels needed for risk assessments of pathogens in biosolids are not available.

- EPA is currently reviewing a standard quantitative microbial risk assessment framework for use in risk assessments of pathogens in water media, which was also designed with biosolids in mind (U.S. EPA, 2009a, ILSI, 2000).

- Dose-response relationships used in risk assessments of pathogens in biosolids have been derived from nonbiosolids studies (e.g., food or water matrix ingestion), and it is unclear to what extent these relationships may be expected to apply to biosolids, particularly for the inhalation pathway.

- The science of biosolids exposure analysis is still under development and studies of the effects of pathogens in biosolids are limited.

- Little information is available to support the elimination of exposure scenarios or pathways from consideration at all sites where biosolids have been applied. Information may support the screening of exposure pathways from consideration at particular sites.

- Bioaerosol emissions from biosolids have been studied most rigorously in Arizona; few data exist for other regions.

- Exposure assumptions vary in existing risk assessments for bioaerosols generated from biosolids.

- Existing risk assessment studies of pathogens in biosolids at specific sites estimate risk of infection rather than risk of disease.

Many of the research and monitoring gaps related to human health risk assessments of biosolids are described in key papers and are summarized in Table A-1. These include aspects of problem formulation, exposure assessment, and effects assessment.

TABLE A-1

Research, Monitoring, Assessment and Modeling Needs Related to Risk Assessment for Land Application of Biosolids

Need	Reference
Hazard Characterization	
New national survey of pathogens in sewage sludge	NRC (2002)
Research on incidence of prions in biosolids	Pepper et al. (2006)
Research to assess utility of additional indicator microoganisms such as *Clostridium perfringens*	NRC (2002)
Research to assess metabolic status of aerosolized pathogens and environmental and biological factors that influence this metabolic state	Pillai and Ricke (2002)
Research to assess potential for pathogen reproduction within bioaerosols	Pillai and Ricke (2002)
New indicators for viruses in biosolids (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Measures of Exposure (quantifying pathogens)	
Improvement (e.g., analytical specificity, sensitivity, accuracy), standardization, validation of detection methods for bacteria, viruses, protozoan parasites, helminth parasites in biosolids	Smith et al. (2005a), NRC (2002), EPA (2003b)
Standardized methods for measuring and characterizing pathogens in bioaerosols	NRC (2002), Pillai (2002)
Molecular, immunological, immuno-magnetic separation and culture techniques for detection of low numbers of pathogens	Smith et al. (2005a)
Standardization and validation of assays for detecting and enumerating waterborne protozoan parasites (*Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Microsporidia*, *Balantidium*, *Giardia* and *Entamoeba*), fecal coliforms, *Salmonella* spp., enteric viruses and helminth eggs in biosolids matrices	Smith et al. (2005a)

TABLE A-1 cont.

Need	Reference
Measurement of occurrence, survival, fate and transport of cysts of protozoans and worms/nematodes, as well as viruses or surrogates with respect to different treatment and land application scenarios	Smith et al. (2005a)
Evaluation of the usefulness of surrogates and models to determine presence or survival of infectious agents before and after treatment and land application	Smith et al. (2005a)
Measurement of antibiotic resistance determinants in bacteria in biosolids	Smith et al. (2005a)
Measurements of post-treatment pathogen concentrations, confirmation that Class B treatment combined with use restrictions result in below-detection pathogen concentrations	NRC (2002), Gerba (2005)
Creation of matrix of virus concentrations in different types of biosolids, by source of sewage sludge and type of treatment (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Measures of Exposure (fate and transport)	
Research on the fate and transport of bioaerosols from land application or spray irrigation	Smith et al. (2005a), NRC (2002)
Better bioaerosol dispersion and viability models	Pillai and Ricke (2002)
Improved bioaerosol samplers that are designed not only for bacterial collection, but also for virus and endotoxin collection	Pillai (2007)
Research to assess transport and fate of viruses in land applied biosolids (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Monitoring of pathogens at various points in the environmental transport process from the biosolids source to the site of exposure	Eisenberg et al. (2004)
Relationships between pathogen survivorship and environmental factors	Eisenberg et al. (2004)

TABLE A-1 cont.

Need	Reference
Development of site-specific atmospheric dispersion models (and research supporting parameter development) to identify appropriate bioaerosol sampling locations depending on micrometeorological conditions	Pillai (2007)
Research on effect of harvest and grazing restrictions on pathogen fate and transport	NRC (2002)
Monitoring to assess potential exposures from runoff from land application of biosolids (judged by cited workgroup to be a medium priority)	Parasite workgroup in Smith et al. (2005b)
Research to assess fate of viruses most resistant to temperature and high pH treatment processes, i.e., hepatitis A and adenoviruses	Pepper et al. (2006)
Monitoring to assess potential for regrowth of *E. coli* O157:H7 after treatment processes	Pepper et al. (2006)
Measurement of fate of *Cryptosporidium* oocysts during treatment and after soil amendment in a variety of environments	Pepper et al. (2006)
Relevance of correlations between indicator and endpoint microorganisms in biosolids to relationships in aerosols	Brooks et al. (2005b)
Measures of Exposure (biotic uptake)	
Research to assess adequacy of 30-day waiting period for grazing following land application of Class B biosolids (judged by cited workgroup to be a medium priority)	Virus workgroup in Smith et al. (2005b)
Measures of Exposure (human parameters)	
Research on exposure of workers and off-site residents to biosolids and biosolids components (bioaerosols, dust)	Smith et al. (2005a) Virus workgroup in Smith et al. (2005b)
Information on actual ingestion and inhalation rates, as well as duration of exposure (e.g., percent of inhaled bacteria that are swallowed)	Gerba and Smith (2005), Brooks et al. (2005b)

TABLE A-1 cont.

Need	Reference
Determination of route of exposure of humans to aerosolized pathogens	Pillai (2007)
Information on household-level transmission of pathogens	Eisenberg et al. (2004)
Information on human transmission of pathogens (such as nontyphi *Salmonella*) by inhalation of bioaerosols and associated dose-response relationships	Pepper et al. (2006)
Dose-Response Relationships	
Development of relationships between ingested doses and severity and duration of effects, including species and subspecies differences in infectivity	NRC (2002)
Validation of animal-derived dose-response relationships for humans	NRC (2002)
Tests of models used to extrapolate dose-response relationships derived at high doses to low doses	NRC (2002)
Development of relationships between treatment process conditions (time, temperature, pH, chemical doses, holding times), pathogen indicator concentrations and maximum acceptable pathogen concentrations	NRC (2002)
Research on the role of chemical irritants in affecting pathogen-related risks	Lewis et al. (2002)
Research on infectivity of aerosolized microbial pathogens, especially enteric pathogens	Pillai and Ricke (2002), Pillai (2007)
Determination of infective doses for parasites	Parasite workgroup in Smith et al. (2005b)
Research on minimum infective doses (minimum number of infectious units required to cause an infection), especially for immunocompromised individuals	Lewis and Gattie (2002)

TABLE A-1 cont.

Need	Reference
Research on how different pathogen strains interact in the development of immunity	Eisenberg et al. (2004)
Risk Assessment	
Quantitative microbial risk assessment methods	NRC (2002)
Sensitivity analyses to determine what critical information is needed to reduce uncertainty in microbial risk assessments	NRC (2002)
Risk assessment of *Ascaris* ova, which requires data on levels of viable ova in biosolids and survival under different environmental conditions (many limits for use of agricultural land after land application of Class B biosolids are determined by survival of *Ascaris* ova)	Pepper et al. (2006)
Risk assessment on Class B biosolids and vectors (e.g., flies) for virus transmission (judged by cited workgroup to be a high priority)	Virus workgroup in Smith et al. (2005b)
Risk assessment for exposure of public to Class B biosolids, including scenarios where food crops are grown or harvested (judged by cited workgroup to be a high priority)	Virus workgroup in Smith et al. (2005b)
Population-based risk model related to biosolids properties and properties of pathogens from biosolids	Eisenberg et al. (2004)
Research on management alternatives such as riparian buffers	Smith et al. (2005a)
Validation of health risk models using epidemiological studies	Pillai and Ricke (2002), Pillai (2007)
Causal Analysis	
Demonstration of causal association between biosolids exposures and adverse health outcomes	NRC (2002)
Framework for establishing causation in human health investigations, including (1) studies in response to unusual exposures and unusual occurrences of disease, (2) preplanned studies to characterize exposures of workers and communities, and (3) epidemiological studies of biosolids use	NRC (2002)

TABLE A-1 cont.	
Need	Reference
Epidemiological studies on exposed populations such as those who apply biosolids including farmers and communities near land application sites	NRC (2002), Dowd et al. (2000)
Rapid response investigations of reported health effects potentially resulting from land application of biosolids	EPA (2003b) from Water Environmental Research Foundation (WERF) Biosolids Research Summit

NRC RECOMMENDATIONS

The NRC was asked by EPA to evaluate "technical methods and approaches used to establish the chemical and pathogen standards for biosolids, focusing specifically on human health protection and not ecological or agricultural issues" (NRC, 2002). NRC recognized the need to reduce uncertainty about potential for adverse human health effects from exposure to biosolids (NRC, 2002).

Many of the committee's recommendations are pertinent to a problem formulation for risk assessment of land application of biosolids. The Committee on Toxicants and Pathogens in Biosolids Applied to Land was asked to perform the following pathogen-related tasks:

- "Review the current standards for pathogen elimination in biosolids and their adequacy for protecting public health. Consider (a) whether all appropriate pathogens were considered in establishing the standards; (b) whether enough information on infectious dose and environmental persistence exists to support current control approaches for pathogens; (c) risks from exposure to pathogens found in biosolids; and (d) new approaches for assessing risks to human health from pathogens in biosolids."
- "Explore whether approaches for conducting pathogen risk assessment can be integrated with those for chemical risk assessment. If appropriate, recommend approaches for integrating pathogen and chemical risk assessments."

Biosolids management practices and recent risk assessment methods were reviewed. The committee reviewed evidence of human health responses to biosolids including anecdotal allegations of disease, reviewed risk assessments and technical data used to develop pathogen standards, and examined management practices of the Part 503 rule. Peer-reviewed literature and government reports on human health effects of biosolids and treated wastewater were reviewed and described in a table in the NRC report, with no attempt to verify other allegations. The committee noted that a cause and effect relationship between biosolids and adverse health effects has not been documented (NRC, 2002) (see Table A-1). Overarching recommendations included: (1) supplementing technological approaches with risk assessments to establish regulatory criteria for pathogens in biosolids; (2) conducting a new national survey of pathogens in sewage sludge; and (3) developing a framework for establishing

causation in human health investigations, including (a) studies in response to unusual exposures and unusual occurrences of disease, (b) preplanned studies to characterize exposures of workers and communities and (c) epidemiological studies of biosolids use NRC (2002, Table A-1). Furthermore, the committee recommended that EPA assess the reliability of biosolids treatment processes, monitor compliance with pathogen standards, conduct environmental hazard surveillance, and study human exposure and health.

More specific recommendations of the NRC committee included the use of new indicator organisms, such as *Clostridium perfringens* in regulation of land application of biosolids (see Table A-1). Moreover, the committee recommended that site restrictions, buffer zones and holding periods for applications of Class B biosolids be specific to geographic and site-specific conditions that affect fate and transport of pathogens. The committee recommends verification of site restrictions to determine if they meet their intended pathogen levels (see Table A-1).

Regarding risk assessment, the committee recommended that a conceptual site model should be used to identify all potential routes of exposure (NRC, 2002). The committee found that it is not yet possible to integrate pathogen risk assessment with chemical risk assessment, given the data gaps and paucity of risk assessment methods for complex mixtures. Furthermore, they noted that several exposure pathways were not adequately addressed in the 1993 Part 503 pathogen requirements, including the inhalation pathway, the potential for surface-water contamination by runoff, groundwater contamination and secondary transmission of disease (NRC, 2002). In particular, pathogen transport and survival in bioaerosols is highly uncertain (see Table A-1). Many of these research, monitoring and assessment gaps are included in Table A-1.

PATHOGENS

Extensive information is available describing pathogens that may be present in Class B biosolids as well as their potential effects. Pathogens include bacteria, enteric viruses, protozoan pathogens, helminths, and others. Articles that provide detailed information on these classes of pathogens include Epstein (2006), Epstein and Moss (2006), Pepper et al. (2006), NRC (2002), Straub et al. (1993) and chapters in Smith

et al. (2005b). The list of potential pathogens is long, but little information is available to eliminate particular agents. However, researchers contributing to the Smith et al. (2005b) volume selected and provided criteria for selecting the most significant bacterial, viral and parasitic pathogens.

Many of the articles above provide information on indicators of pathogens in biosolids. Dowd et al. (1997) recommend thermotolerant clostridia as indicators of fecal contamination in bioaerosols. Pillai et al. (1996) found that clostridia and H_2S (hydrogen sulfide) producers were better indicators of airborne biosolids-derived material than traditional bacterial indicators (fecal coliforms and fecal streptococci).

The primary information gap related to hazard characterization is recent national-scale data on the distributions of concentrations of pathogens in biosolids, with respect to method of treatment, acceptable analytical methods for detecting and quantifying pathogens and other variables (see Table A-1). Epstein and Moss (2006) cite references regarding probable numbers of fecal coliforms and *Salmonella* spp. in Class B biosolids. Dahab and Surampalli (2002) found that existing treatment systems do achieve Class B requirements under the U.S. Part 503 rule, while Class A may not be easily achieved.

Biosolids experts distinguish between traditional and emerging pathogens, and Gerba et al. (2002) reviewed the latter. A committee of experts convened at the Workshop on Emerging Infectious Disease Agents and Issues associated with Sewage Sludge, Animal Manures and Other Organic By-Products in Cincinnati, OH, June 2001, concluded that emerging pathogens do not exhibit survival or other properties that are very different from those exhibited by traditional pathogens (Smith et al., 2005a). Pepper et al. (2006) reviewed studies of various traditional and emerging pathogens and summarized which have been detected in biosolids and which have not been detected in biosolids or not studied.

One recent study found that biosolids were not a likely source of *Staphylococcus aureus* exposure or infection (Rusin et al., 2003a). Helminths are probably the most persistent of enteric pathogens (Pepper et al., 2006; Straub et al., 1993). Little research on the survival of protozoan parasites (e.g., *Cryptosporidium* species, *Giardia*) in biosolids-amended soil has been conducted.

It is impossible to test biosolids for all possible pathogens (Smith et al., 2005a). Enteric viruses and helminth ova have been selected as indicators of treatment efficacy because they are resistant to treatment and can be quantified (Smith et al., 2005a).

Chapter 4 in Smith et al. (2005b) provides detection/analytical capabilities and recommendations for bacterial pathogens in biosolids.

MEASURES OF EXPOSURE

Numerous factors determine human exposure to pathogens in biosolids. These include health status of contributors, method of treatment, percent solids, friability, exposure to heat and ultraviolet. The literature search did not include an exhaustive search for articles on factors that influence the fate of pathogens. The review below presents a sampling of articles on the topic.

Detection of Pathogens

The detection of pathogens in environmental samples such as biosolids-amended soil is inefficient. For example, Rusin et al. (2003a) had a recovery efficiency of 8.7% for *Staphylococcus aureus* in Class B biosolids. Organic matter and high bacterial counts reduce recovery fraction for pathogens (Rusin et al., 2003b).

Decay of Pathogens

Lang et al. (2003) studied the decay of *E. coli* in biosolids-amended sandy loam soil and quantified indigenous *E. coli* in control soils in the United Kingdom. Stine et al. (2005) studied survival of bacterial and viral pathogens on the surface of fruit and vegetable crops, but not in a biosolids matrix. Straub et al. (1993) reviews studies of survival of pathogens in soil and sewage sludge.

Lewis and Gattie (2002) assert that models typically use data from experiments from enteric organisms such as *E. coli* and *Salmonella* to estimate bacterial survival rates. They point out that these microorganisms are short-lived compared to those that form spores or are encapsulated (such as *Mycobacterium* spp.).

Gerba et al. (2002) investigated which emerging pathogens are likeliest to survive Class B biosolids treatments. Literature was reviewed (1) relating pathogen

survival to temperature and environmental variables, (2) documenting pathogen occurrence in biosolids and (3) describing dose-response models for pathogens. The study concluded that adenoviruses and hepatitis A were heat resistant viruses and therefore likely to survive long periods in the environment. *Escherichia coli* O157:H7 and *Listeria monocytogenes* are emerging bacterial pathogens that can survive anaerobic digestion and can sometimes regrow following land application of biosolids. In contrast, the parasites microsporidia and *Cyclospora* would not survive under high temperatures of anaerobic digestion or under conditions of low moisture.

Reactivation and Regrowth of Pathogens

Zaleski et al. (2005a) asked "Does regrowth occur following reintroduction or recolonization of pathogens after land application or during storage under favorable conditions?" The authors note that regrowth of indicator bacteria and *Salmonella* in biosolids has been observed under certain moisture, temperature and substrate conditions, and when indigenous bacteria are low. Moreover, pathogens in biosolids may be reduced if they are stored at certain moisture and temperature ranges. In biosolids-amended soils, increased moisture may lead to survival and regrowth of bacterial pathogens. In one study the use of concrete-lined beds for storage during desiccation allowed moisture from rainfall to accumulate in the beds, leading to growth of fecal coliforms and salmonellae added from external sources (Zaleski et al., 2005b). Furthermore, survival rates of bacteria are higher in soil of finer textures (Zaleski et al., 2005a).

Aerial Transport of Pathogens

Pathogens have rarely been measured in biosolid aerosols (see Table A-1). Pillai and Ricke (2002) reviewed factors controlling bioaerosol transport, as well as bioaerosol sampling methods and culture-based approaches to the detection and characterization of specific components of bioaerosols.

Brooks et al. (2004a) measured bioaerosol emissions during land application of Class B biosolids in the region of Tucson, AZ. The objective was to develop empirical models of the fate and transport of bioaerosols. Pathogens and indicator bacteria were

only rarely found in aerosolized samples. These included coliforms and coliphages, which were present at high densities in biosolids, and animal viruses, which were not detected in biosolids. *Clostridium perfringens* was detected only in a small fraction of aerosol samples, but these were present under various weather conditions. The authors suggest that only microorganisms in the aqueous phase of biosolids were able to aerosolize; others remained sorbed to the solid phase (Brooks et al., 2004a).

In another study, Brooks et al. (2006) measured aerosolized endotoxin concentrations downwind of a single biosolids-amended site. Levels were generally within limits previously proposed in occupational exposure studies, though peak concentrations occasionally exceeded these limits. Levels of endotoxin in aerosolized soil were sometimes above those associated with biosolids amended-soil, calling into question whether biosolids were the primary source of the endotoxin. Additional studies of bioaerosol transport that included a risk assessment component are described in the section on risk assessment.

Tanner et al. (2005) determined bioaerosol emission rates and plume characteristics during spray application of liquid Class B biosolids. They did not detect coliphages or coliform bacteria just downwind of the biosolids application (approximately a 2-m distance away), though bacteria that had been added to groundwater and sprayed were detected. The researchers concluded that the presence of biosolids reduces aerosolization of microorganisms relative to application of inoculated groundwater. Even if bacteria had been present below detection limits, the duration of exposure to any pathogens just downwind of biosolids application would be expected to be brief because of the moving applicator (Tanner et al., 2005).

Paez-Rubio et al. (2006) investigated the content of bioaerosols produced during the disking of biosolids on an application site in Central Arizona. Biosolids source emission factors (number of microorganisms or mass of biotoxins per area) and emission rates (number of microorganisms or mass of biotoxins per time) were measured for total bacteria, culturable heterotrophic bacteria (heterotrophic plate counts [HPC]), total coliforms, sulfite-reducing *Clostridia*, and endotoxin, as well as particulate matter 10 micrometers (PM_{10}). The authors presented a correlation between microbial concentrations emitted during disking and their content in biosolids. Disking was

determined to be a “substantial source of biosolids-derived aerosols” and might be of greater potential concern than other application methods. The emission rate during disking of biosolids was greater than rates that had been measured during spreading of dewatered biosolids by side slinger or spraying of liquid biosolids. For example, total coliform emissions during disking were about two times greater than emissions associated with spreading dewatered biosolids and at least two orders of magnitude greater than maximum emission rates reported by Tanner et al. (2005) during spraying of liquid biosolids (Paez-Rubio et al., 2006). The authors provide a framework for reconstructing aerosol concentrations and emission rates.

In a related study, Paez-Rubio et al. (2007) measured bioaerosol emission rates from the spreading of Class B biosolids with a side-slinging applicator in Arizona. Concentrations of pathogens in bioaerosols were reconstructed from concentrations in bulk biosolids and PM_{10}. Aerosol emission rates of several bacterial indicators were correlated with their concentrations in bulk biosolids. Aerosol emission rates of dewatered biosolids were one to two orders of magnitude higher than those reported for liquid biosolids. Diameters of emitted particles suggest that most were inhalable and possibly respirable. The authors assert that their work “move[s] aerosol studies beyond indicator measurements by estimating specific toxic compound or pathogen aerosol concentrations based on more easily obtained PM_{10} measurements and bulk biosolids analysis—where detection limits are much lower due to the large sample size possible.” J. Peccia, one of the authors, notes that rates of recovery of pathogens in aerosols that are reported in the literature are currently only about 10% (Lubick, 2007). The authors acknowledge that the relationship between source emission rates and bulk biosolids concentration that they present is limited to the type of spreader they used (i.e., a “ProTwin Slinger” side discharge spreader, the most common spreader for biosolids of the 20−30% solids content range).

Leaching to Groundwater

A review of the literature has concluded that few pathogens from biosolids leach to groundwater (Pepper et al., 2006). For example, Chetochine et al. (2006) measured the numbers and leaching potential of coliphage MS-2, specific to *E. coli*, from Class B

biosolids. Much of the phage was sorbed to or associated with solid particles. Following serial extraction, less than 8% of the phage initially present in the biosolids leached from biosolids-amended soil. The phage was not appreciably retained in a column containing a sandy porous medium. Horswell et al. (2010) tested soil cores and concluded that environmental contamination by virus is unlikely because of strong soil adsorption.

Y. Jin, J. Sims and K. Kniel of the University of Delaware were awarded a U.S. Department of Agriculture (USDA) grant from 2006–2009 to study the fate and transport of viruses in biosolids and their potential to contaminate groundwater and foodcrops as a result of land application of biosolids (Jin et al., 2006).

Erosion and Surface Runoff

The literature search did not find information on these mechanisms of transport of pathogens in biosolids.

Pathogens on Crops

Studies of pathogens on crops are described in the section on risk assessment. Also, the DA grant described above that was awarded to Y. Jin, J. Sims and K. Kniel of the University of Delaware includes an investigation of the contamination of crops (Jin et al., 2006).

RISK ASSESSMENT

Risk Assessment Process

Risk assessments of pathogens in biosolids have been performed by various investigators, but the emphasis has been on the use of particular transport models to quantify exposure and risk, rather than the process of planning and conducting a broad risk assessment. One recent risk assessment of biosolids application found that the science of assessing risk from environmental exposure to biological agents, as well as acceptable levels is “under development at the present time” (Willert and Eng, 2005). Therefore, the focus of that study was altered from the quantification of risk to the effectiveness of a pelletization process to destroy biological agents of potential concern.

Soller et al. (2006a) described general methods for conducting health risk assessments of pathogens in biosolids that were developed as part of a Water Environment Research Foundation project. The methods included characteristics of an infectious disease process, including the consideration of multiple transmission pathways, disease transmission models, and the presence of immunity (Soller et al., 2006a,b). Soller et. al.'s framework for evaluating human risks associated with microbes in biosolids included an exposure characterization component (quantifying pathogen levels in the environment) and a health effects component. A schematic diagram displayed several Class A and Class B sludge treatment processes as well as environmental variables affecting exposure (time, temperature and moisture). They described the tradeoff between site-specific monitoring data and more general data on treatment effectiveness and fate and transport of pathogens from points earlier in the waste stream. A conceptual health effects model was also included in the report. This model, first published in Eisenberg et al. (2004), contained six epidemiological states: (1) susceptible state, (2) exposed state (asymptomatic and infectious), (3) carrier state 1 (asymptomatic but infectious), (4) diseased state, (5) carrier state 2 (previously symptomatic, now asymptomatic and infectious) and (6) protected state (postinfectious and noninfectious and some level of immunity). Soller et al. (2006a) also included a table of data required to parameterize a basic health effects model.

Although Soller et al. (2006a) included information and diagrams useful for developing a problem formulation for pathogens in biosolids, they did not organize it as a problem formulation. These elements are found in the *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998).

The International Life Sciences Institute developed a framework for microbial risk assessment related to human exposures and waterborne pathogens (ILSI, 2000). The framework describes the stages of risk assessment, including problem formulation, but does not provide or cite scientific advice regarding particular pathogens or exposure pathways.

Pathways for Bioaerosol Exposure

One of the primary research needs identified by the NRC was human exposure to pathogens in bioaerosols (NRC, 2002). Researchers at the University of Arizona conducted several major studies to help understand community and worker risk of infection from bioaerosols, as well as to develop methods for modeling transport of pathogens and human exposure (Brooks et al., 2004a, 2005a,b, 2006, Tanner et al., 2008). Prior to that study, the same group of researchers studied bioaerosols in West Texas (Dowd et al., 2000). Conclusions were that community risks were relatively negligible, with worker risks somewhat higher.

Dowd et al. (2000) sampled bioaerosols emitted from anaerobically digested, dewatered biosolids applied in west Texas. The study generated bacterial and virus release rates from large biosolids piles where they were stored prior to application and fields where biosolids were sprayed. Levels of *Salmonella* and an indicator virus (coliphage) were measured. The ratio between the concentration of indicator virus in aerosols and the concentration in biosolids was used to estimate a value for airborne enteric virus (Coxsackievirus). Microbial transport models (a point source model and an aerial source model) were used to generate downwind concentrations. Dose-response models were used to estimate risk to workers on site and nearby residents at least 10 km away. The pathway was assumed to consist of inhalation and swallowing of the pathogen. The single hit exponential model [$p = 1 - \exp(-rN)$] was used to describe the probability of infection by Coxsackievirus B3, and the Beta-distribution model ($p = 1 - [1 + (N/\beta)(2^{1/\alpha}-1)]^{-\alpha}$) was used to describe the risk of infection by *Salmonella* serovar Typhi, where p = probability of infection, N = number of organisms inhaled, β is the ID_{50}, and α and r are parameters that describe the dose-response curve. The authors indicated that several sources of conservatism must be considered when evaluating these risk estimates (e.g., the wind does not always come from the same direction, Dowd et al., 2000). Brooks et al. (2004b) corrected a mathematical error in Dowd et al. (2000) which resulted in the newly calculated risk estimates being much lower than reported in Dowd et al. (2000). Citing comments by Brooks et al. (2004a) on the improved efficiency of modern wastewater treatment plants, Pepper et al. (2006)

argue that a more realistic estimate of infectivity is five orders of magnitude lower than Dowd's worst case estimates.

Brooks et al. (2005b) undertook a study to estimate risks of microbial infection of residents near biosolids application sites. At 10 sites throughout the United States that were amended with either liquid or solid Class B biosolids (five sites in Arizona, two in Washington State, one in Virginia, one in Texas and one in Illinois), they measured HPC bacteria, total coliform bacteria, *E. coli*, *Clostridium perfringens*, coliphage, enteroviruses, hepatitis A virus and norovirus in aerosol samples downwind from application sites. The study distinguished between loading, unloading, land application and background operations. In general, risks of infection were determined to be low, with the greatest risk of infection, 4×10^{-4}, from coxsackievirus A21 released during loading operations.

Brooks et al. (2005b) cited a dissertation of Tanner (2004) in reporting that the risk of infection to a biosolids handler can reach as high as 34% annually from exposure to coxsackievirus A21 and 2% annually from exposure to *Salmonella* species. This study assumed exposure on a daily basis (250 days per year).

Brooks et al. (2005a) developed an empirical transport model for viruses aerosolized during land application of liquid biosolids. Data were generated from collections of bioaerosols in field tests with coliphage MS-2 added to water and sprayed with a biosolids spray application truck. Risks of infection for residents adjacent to land application sites were also calculated at 10^{-7} (realistic) to 10^{-5}. Conservative annual risks were calculated at no more than seven times that value. A second goal of the study was to develop a transport model for bacteria, but *E. coli* used in the study did not typically survive the aerosolization process.

Based on Brooks' studies, Pepper et al. (2006) concludes that overall community risk of infection from bioaerosols during land application was relatively negligible. Occupational risks during land application were higher than community risks but were still low (Brooks et al., 2004). Pillai (2007) cautions against extrapolating these results to different source materials, regions, or even parts of a region. Pathogens in biosolids might be more desiccated or inactivated from exposure to ultraviolet light than in other parts of the country.

In a study of bioaerosol emission rates from the spreading of Class B biosolids in Arizona, measured source endotoxin concentrations that were greater than reported conservative thresholds for mucous membrane irritation, and most exceeded the threshold for acute bronchial constriction (Paez-Rubio et al., 2007).

Pathways for Groundwater Contamination

Based on a review of the literature such as Chetochine et al. (2006, above), Pepper et al. (2006) conclude that groundwater contamination from land-applied biosolids is not likely, and therefore human health risks are likely negligible. By extension, pathways by which pathogens in groundwater may contaminate land or surface water via springs or other interactions are also unlikely to be significant for pathogens from biosolids.

Ingestion of Soil

Gerba et al. (2002) used a beta-Poisson model ($P = 1 - [1 + N/\beta-\alpha]$) from Haas et al. (1999) to assess the risk of infection and illness from enteric viruses following land application of Class B biosolids, assuming that exposure was from ingestion of biosolids-amended soil. They focused on rotavirus and echovirus 12. Gerba et al. (2002) determined that direct ingestion of biosolids, if they were spread across the surface of the soil, would result in an annual risk from a one time exposure exceeding 1×10^{-4}. They assumed no natural attenuation of virus. Injection of biosolids into the soil results in a risk below this level.

Consumption of Vegetation

Most of the information on risks from the crop ingestion pathway is from the United Kingdom. Consumption of root crops is assumed to represent the worst case scenario because they contain higher proportions of soil than leafy crops and they are often consumed uncooked (Gale, 2005a). Gale (2003) estimated the exposure of root crops to *Cryptosporidium* and *Salmonella* species from biosolids applied to agricultural land in accordance with the United Kingdom's Safe Sludge Matrix. An approach using event trees combined with empirical data was used to estimate pathogen levels in raw

sewage sludge, in treated sludge and biosolids mixed with topsoil and root crops. Expert opinion suggested that up to 2% of root crops by weight may be soil at the point of harvest. Monte Carlo simulations were performed to model variation in *Salmonella* levels on root crops, assuming a Poisson-log-normal distribution of bacterial counts.

Gale (2005b) conducted risk assessments to estimate the number of humans in the United Kingdom at risk from consumption of root crops obtained from areas where biosolids were applied according to the Safe Sludge Matrix regulations. (Gale [2005a] presents a subset of that study.) Seven classes of pathogens were the focus of the study: salmonellas, *Listeria monocytogenes*, campylobacters, *Escherichia coli* O157, *Cryptosporidium parvum*, *Giardia* and enteroviruses. The study showed that if linear decay were assumed to occur and if the treatment process (mesophilic anaerobic digestion [MAD]) were assumed to be 100% efficient, potential risks from the seven classes of pathogens were essentially eliminated. If pathogen decay in treated soil was assumed not to occur, then 50 *Giardia* infections were expected in the United Kingdom and less than one infection per year resulting from the other six pathogens. Also if the MAD process was 99% or lower, substantially more infections from *Giardia* and possibly *E. coli* O157 were predicted.

Gale and Stanfield (2001) calculated risks to humans from consumption of vegetable crops contaminated with the bovine spongiform encephalopathy agent in sewage sludge in the United Kingdom. Hinkley et al. (2008) suggest that prions survive wastewater treatments and Pepper et al. (2006) identified the incidence of prions in biosolids as a research priority in the United States (see Table A-1).

Proliferation of Antibiotic Resistance

In addition to risks to human health from specific pathogens, another relevant indirect health issue is the possible proliferation of antibiotic resistant bacteria. The potential risk is that human pathogenic strains become resistant to overused antibiotics, which can no longer treat the pathogen. Pepper et al. (2006) ask the question “Can antibiotic resistant genes be transferred from nonpathogenic bacteria to human pathogenic strains?” Brooks et al. (2004a, 2007a) concluded that Class B biosolids had an equal or lower incidence of antibiotic resistant bacteria compared to unamended soil.

The NRC (2002) did not “believe that land-applied biosolids have any substantial potential to alter the prevalence of antibiotic resistance among pathogenic organisms.”

Infectivity

Gerba and Smith (2005) describe broad risk assessment principles for land application of wastes based on a quick review of the literature, as well as their own experience and expertise. They note that information on infectivity of enteric pathogens is available from many human feeding or inhalation studies.

Dose-response data suggest that a threshold infectious dose does not exist for enteric pathogens (Gerba and Smith, 2005). Infectivity of enteric viruses is greater than infectivity of enteric bacteria. Of known human enteric viruses, rotavirus is the most infectious, causing 10−15% of those ingesting the virus to become infected. Half of the people infected with an enteric pathogen become ill. Mortality is typically less than 1%, but greater for infants, young children, the elderly and immunocompromised people (Gerba and Smith, 2005). A recently developed norovirus dose-response model by Teunis et al. (2008a) includes the above enteric virus characteristics and incorporates additional parameters that may be used for enteric virus risk assessments.

Nwachuku and Gerba (2004) address the susceptibility of children to pathogens, including increased sensitivity and increased exposure. Other reasons why children are at greater potential risk from pathogens in biosolids include the following:

- immature immune system;
- intestinal mucosa more permeable to water;
- proportionally less extracellular fluid than adults;
- physiological deficiency in Immunoglobulin A; and
- reduced stomach acid and pepsin secretion.

For example, children appear to be the most sensitive population to enteroviruses. Studies have not been conducted to estimate relative infectivity of

enteric pathogens for children and adults. However, reduced stomach acid and pepsin secretion could make children more likely to be infected than adults for a given dose.

Disease Risk

Existing empirical studies of biosolids do not estimate disease risk. However, risks of disease might be assumed to be 10% that of infectious risk, though this quantity varies with microorganism (Haas et al., 1999). Soller and Eisenberg (2008) provide parameter values for the proportion of the infected individuals with symptomatic responses for enteroviruses, rotavirus, *Cryptosporidium*, *Giardia lamblia*, Salmonella, *E. coli* O157:H7, Shigella, and a composite value (minimum of 10, median of 40, maximum of 75%).

Dynamic Risk Model

In assessing microbial risks, one may choose either a static or a dynamic risk model (Soller et al., 2008; U.S. EPA, 2006a). Eisenberg et al. (2004) developed a deterministic, dynamic model for estimating risks from pathogens in biosolids. In addition to infectivity, their model considered person-to-person transmission, immunity, asymptomatic infection and incubation period. The model contains six disease states: (1) susceptible state, (2) exposed state (asymptomatic and infectious), (3) carrier state 1 (asymptomatic but infectious), (4) diseased state, (5) carrier state 2 (previously symptomatic, now asymptomatic and infectious) and (6) protected state (postinfectious and noninfectious and some level of immunity). Processes that were not accounted for include climate, behavior and various environmental factors that are not well understood. Three types of risks were estimated: individual-level single event risk, individual-level annual risk and population level attributable risk (Eisenberg et al., 2006). The model was demonstrated in a case study involving the direct ingestion of enterovirus. Sensitivity analysis of simulations in the case study showed that the four most important factors in determining the risk attributable to biosolids were: (1) the relative contribution of biosolids toward exposure, relative to other pathways; (2) the rate of pathogen shedding by infectious people; (3) the rate of person-to-person transmission and (4) immunity. Risk attributable to biosolids was “low” if the rate of

pathogen shedding was relatively high or low or if person-to-person transmission was relatively "high." These were not necessarily intuitive results. The simulations resulted in a decision tree for classifying risk associated with biosolids as high or low.

EXPOSURE ASSUMPTIONS

EPA does not have standard exposure factors for use in risk assessments of pathogens in biosolids. Risk assessment results described above are highly dependent on human exposure factors, and these vary from study to study. For example, because human transmission of aerosols containing *Salmonella* has not been demonstrated, researchers make different assumptions about the percentage of inhaled particles that would be ingested. Pepper et al. (2006) describe studies that use 10%, and Brooks et al. (2005b) uses 50%.

Very little information is available that would allow us to compare the relative importance of different exposure pathways. Academic studies tend to emphasize a single exposure pathway rather than a comparison of multiple pathways. Many studies have found low risk. For example, a British study by Gale (2005b) concluded that risk to human health from consumption of vegetation crops contaminated with pathogens in biosolids is low. Moreover, a study of bioaerosols in Arizona found that risk of infection of residents from bioaerosols generated during land application of biosolids was rather negligible at 10 km, though if residents were assumed to reside closer, estimated risks would have been higher (Brooks et al., 2005b; Pepper et al. 2006). Based on a review of the literature, Pepper et al. (2006) conclude that "groundwater contamination from land-applied biosolids does not appear to be likely." Moreover, it is argued that regrowth of pathogens in biosolids-amended soil may be ignored because of the biological competition in Class B biosolids (Pepper et al., 2006; Zaleski et al., 2005a,b). However, insufficient information is available to ignore particular exposure pathways at all sites.

CAUSAL ANALYSIS

"Causal association between biosolids exposures and adverse health outcomes has not been documented" (NRC, 2002). Gattie and McLaughlin (2004) investigated

public complaints and concluded that irritants associated with volatile chemicals and dust blowing from biosolids treated land (e.g., bacterial toxins, lime, organic amines) may cause nearby residents to be more susceptible to infections. Lewis et al. (2002) recorded symptoms reported by 48 residents near 10 biosolids application sites in the United States and Canada. The wide range of symptoms included various combinations of coughing, burning eyes, sore throat, burning lungs, headache, congestion, difficulty breathing, flu-like symptoms, fever, nausea/vomiting, diarrhea, sinusitis, staphylococcal infection, pneumonia, skin rash, nosebleed and fatigue. The researchers did not establish cause and effect between biosolids and reported adverse effects. They suggested that chemical contaminants in biosolids might irritate the skin and mucous membranes and thus increase pathogen host susceptibility (Lewis et al., 2002). Another survey conducted by Khuder et al. (2007) suggests a higher risk of certain respiratory, gastrointestinal, and other diseases among residents living near farm fields on which biosolids were applied.

In contrast, Dorn et al. (1985) conducted a health effects study of 47 biosolids application sites (annual applications) and 46 control sites on farms in Ohio. Estimated risks of respiratory illness, digestive problems or other general symptoms did not differ between biosolids and nonbiosolids farms. The authors cautioned readers when considering the results in the context of larger acreages, higher application rates or biosolids containing larger concentrations of pathogens.

NRC (2002) summarized studies of sewer workers and others exposed to raw sewage to identify potential hazards from biosolids. The committee also summarized a survey study in which workers who loaded, unloaded and applied Class B biosolids had a history of gastrointestinal illness. However, it was later determined that the biosolids did not meet Class B requirements.

Simmonds et al. (2005) describe the difficulties of conducting an epidemiological study of biosolids exposure. Few people who are exposed are expected to become infected, and even fewer to manifest symptoms of disease. Also, various symptoms may be associated with one pathogen, and various pathogens can cause similar symptoms.

Preliminary work has been done to scope epidemiological designs to assess acute health effects and community-level exposure to treated sewage sludge (Class B biosolids) in North Carolina and Virginia (Heaney et al., 2006).

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