

Protocol for Microbial Risk Assessment
to Support Human Health Protection
for Water-Based Media

DRAFT

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Notice

The policies and procedures set forth in this document are intended solely to describe EPA's protocol for conducting or revising microbial risk assessments to protect human health from exposure to water-based media. They are also intended to serve as guidance to EPA and EPA contractors for conducting microbial risk assessments.

This document has not yet been reviewed in accordance with Agency policy and approved for publication and distribution.

Mention of commercial products, trade names, or services in this document or in the references and/or endnotes cited in this document does not convey, and should not be interpreted as conveying, official EPA approval, endorsement, or recommendation.

Foreword

This document presents the U.S. Environmental Protection Agency's (EPA's) recommended MRA Protocol for planning and conducting microbial risk assessments (MRAs) in support of human health protection for water-based media. The MRA Protocol provides guidance for microbial risk assessments conducted or revised by EPA or EPA contractors and should not be considered regulatory.

This MRA Protocol is focused on conducting risk assessment for water-related media (such as microorganisms in treated drinking water, source water for drinking water, recreational waters, shellfish waters, and biosolids), but is sufficiently general to help guide the development of microbial risk assessments of pathogens that might be found in food, food products, or other media.

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The International Life Sciences Institute Risk Science Institute (ILSI RSI) under cooperative agreements with the EPA Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, developed a report titled *Revised Framework for Microbial Risk Assessment* to address critical areas of microbial risk assessment (ILSI, 2000). This MRA Protocol is based primarily on that report. The steering committee for the development of that report included the following:

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This document has been subject to external peer review following OMB's Peer Review Guidance and EPA's Peer Review Handbook.

Potential areas for conflict of interest have been investigated via direct inquiry with the potential peer reviewers and review of their current and past affiliations. Reviewers did not have conflicts of interest.

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Acronyms

| | |
|------------------|---|
| AF | adjustment factor |
| AGI | acute gastrointestinal illness |
| AIC | Akaike Information Criterion |
| AIDS | acquired immune deficiency syndrome |
| ALOP | appropriate level of protection |
| ANOVA | analysis of variance |
| ARS | USDA Agricultural Research Service |
| ARS | adaptive rejection sampling |
| AWQC | Ambient Water Quality Criteria |
| BAF | bioaccumulation factor |
| BenMAP | EPA's Environmental Benefits Mapping and Analysis program |
| CAC | Codex Alimentarius Commission (Codex) |
| CART | classification and regression tree |
| CDC | U.S. Centers for Disease Control and Prevention |
| CEA | cost effectiveness analysis |
| CCL | Contaminant Candidate List |
| cfu | colony forming units |
| CSFII | Continuing Survey of Food Intake by Individuals |
| CWA | Clean Water Act |
| DALY | disability-adjusted life years |
| DNA | deoxyribonucleic acid |
| DSA | differential sensitivity analysis |
| EIP | CDC's Emerging Infections Program |
| EJ | environmental justice |
| EMPACT | Environmental Monitoring for Public Access and Community Tracking |
| EPA | U.S. Environmental Protection Agency |
| ERA | ecological risk assessment |
| FAO | Food and Agricultural Organization (United Nations) |
| FDA | U.S. Food and Drug Administration |
| ffu | focus forming units |
| FoodNet | Foodborne Diseases Active Surveillance Network |
| FUT2 | alpha (1,2) fucosyltransferase gene |
| GI | gastrointestinal (tract) |
| GIS | graphical information system |
| HIV | Human Immunodeficiency Virus |
| ICR | Information Collection Rule |
| ID ₅₀ | infectious dose for 50% of the exposed population |
| IgG | immunoglobulin |
| ILSI | International Life Sciences Institute |
| L | liter |
| LD ₅₀ | lethal dose for 50% of the population |
| LT2 | Long Term 2 Enhanced Surface Water Treatment Rule |
| MAC | <i>Mycobacterium avium</i> complex |

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| MCMC | Markov Chain Monte Carlo method |
| MF | modifying factor |
| MILY | Morbidity Inclusive Life Years |
| mL | milliliter |
| MLE | maximum likelihood estimation |
| MOS | margin of safety |
| MRA | microbial risk assessment |
| MRM | microbial risk management |
| NAS | National Academy of Sciences |
| NCRP | National Committee on Radiation Programs |
| NGO | nongovernmental organization |
| NRC | National Research Council |
| NRCS | Natural Resources Conservation Service |
| NRSA | nominal range sensitivity analysis |
| NV | Norwalk Virus |
| OAQPS | Office of Air Quality Planning and Standards |
| OMB | Office of Management and Budget |
| PCR | polymerase chain reaction |
| pfu | plaque forming unit |
| PMP | Pathogen Modeling Program |
| PPBK | physiologically-based biokinetic (modeling) |
| PWS | public water supply |
| QALY | quality-adjusted life years |
| QMRA | quantitative microbial risk assessment |
| RSI | Risk Science Institute (ILSI) |
| RT-PCR | reverse transcriptase polymerase chain reaction |
| SAB | Science Advisory Board (EPA) |
| SDWA | Safe Drinking Water Act |
| SDWIS | Safe Drinking Water Information System |
| SF | safety factor |
| SMV | Snow Mountain Agent Virus |
| SPS | sanitary and phytosanitary |
| TCCR | transparency, clarity, consistency, and reasonableness |
| TMDL | total maximum daily load |
| UF | uncertainty factor |
| U.S. | United States |
| USDA | U.S. Department of Agriculture |
| UV | ultraviolet (light) |
| VBNC | viable but non-culturable |
| WQ | water quality |
| WHO | World Health Organization (United Nations) |

Executive Summary

Exposure to waterborne pathogens has long been recognized as a potential source of illness in humans. Managing and minimizing this public health threat is an important aspect of the United States (U.S.) Environmental Protection Agency's (EPA) Office of Water regulatory activities and policy development. Risk assessment is a science-based tool that can be used to help managers explore the relative merits of various management alternatives, identify important gaps in knowledge, and inform regulatory actions.

This Microbial Risk Assessment (MRA) Protocol was developed as guidance to assist EPA and others in conducting MRAs that are well documented and are respected by the scientific community. The primary audience for this document is EPA staff and contractors who are responsible for conducting and managing MRAs for hazards that occur in water and water-related media. Thus, this document is intended to: provide guidance to risk assessors and scientists; summarize MRA methods and techniques; and provide a compilation of information that is useful for conducting rigorous and scientifically defensible MRAs. It is not however, intended to be a comprehensive treatise nor a textbook on the topic of MRA. Although the principle medium of interest is water and water-related media (e.g., recreational waters, drinking water sources, shellfish harvesting waters, biosolids), resources for food safety risk were also consulted in the development of this MRA Protocol.

This Protocol should be considered flexible and amenable to modification where an Office or other user has particular requirements that may not be precisely covered in the text. Moreover, this guidance should be considered a modular tool box with a broad scope. It is expected that those modular aspects that are relevant to the MRA being conducted can be used as deemed appropriate by the EPA Office conducting the assessment. This Protocol does not include discussion and evaluation of ongoing state-of-the-art research to support the field of MRA.

Microbial risk assessments can be initiated for a variety of reasons, including but not limited to the following:

- to assess the potential for human risk associated with exposure to a known pathogen;
- to determine critical points for control, such as watershed protection measures;
- to determine specific treatment processes to reduce, remove, or inactivate various pathogens;
- to predict the consequences of various management options for reducing risk;
- to determine appropriate criteria (regulatory) levels that will protect individuals and/or populations to a specified risk level or range
- to identify and prioritize research needs; and
- to assist in interpretation of epidemiological investigations.

Individual risk assessments for specific situations can differ significantly with respect to the questions that are addressed, the information required to address those questions, and the nature of data gaps.

This MRA Protocol is comprised of a combination of concepts from numerous published risk assessment frameworks and workshop proceedings. Although many of these frameworks and proceedings were originally developed for other applications such as food safety, there are many principles that also apply to water-related risk assessments. This MRA Protocol employs primarily an expanded and enhanced version of the EPA/ILSI Framework for MRA. For the purposes of this document, the EPA/ILSI structure has been modified in the recognition that MRA practitioners and managers often desire flexibility in the development of MRAs, and that in some cases the National Academies of Sciences, National Research Council (NRC) chemical risk framework (or another framework) may be preferred. A common theme among frameworks is the iterative nature of risk assessment. The modeling steps in risk assessment may be repeated multiple times as the scope of the assessment is refined or as risk management questions evolve. Additional data and sensitivity analyses also require repeated iterations.

Chapter 1 provides an introduction to MRA and summarizes concepts that are used throughout this document, including the purpose and scope of this document and background information. It also includes an overview of appropriate frameworks for the conduct of MRAs, such as the framework developed by the NRC in 1983 and previous EPA guidance. Chapter 2 describes Problem Formulation and Planning and Scoping; it includes an outline that can be used for problem formulation documentation and a description of how problem formulation can be used to track the risk assessment progress and process. Hazard identification is also discussed in Chapter 2 as one critical component of the problem formulation process. Chapter 2 corresponds to both Phase I and the iterative parts of Phase II of the 2008 NRC framework.

Chapters 3 and 4 describe the analysis phase of MRA, which consists of two separate but related components—characterization of exposure and characterization of human health effects. Exposure characterization is discussed in Chapter 3 while the characterization of human health effects, including the dose-response assessment, is the focus of Chapter 4. Subtopics within the characterization of exposure include the occurrence of the infectious disease hazard, exposure analysis, and the exposure profile (a summary of the results of the exposure characterization process). Subtopics within characterization of human health effects include description of health effects, dose-response relationship, and the host-pathogen profile (summary of the results of the characterization of health effects). Common forms of dose-response models are also summarized.

Chapter 5 discusses the risk characterization phase of MRA. The topics summarized include the historical context of risk characterization within EPA, a discussion of risk assessment model parsimony, an overview of commonly used MRA model forms, and a summary of methods to represent the data used in MRA models. Uncertainty analysis and sensitivity analysis are discussed within the context of risk characterization. Collectively, Chapters 3, 4, and 5 correspond to Phase II (Stage 2) of the 2008 NRC framework. Chapter 5 also includes some of the concepts included in Phase III of the 2008 NRC framework.

A total of seven appendices (A-G) are also included with this MRA Protocol. The purpose of the appendices is to provide interested readers with additional detail or background on topics included in the Protocol.

1. Introduction

1.1 Purpose and Scope of this MRA Protocol

The primary purpose of this document is to provide guidance to U.S. Environmental Protection Agency (EPA) staff and contractors for conducting microbial risk assessment (MRA) for hazards that occur in water and water-related media. Although the principle medium of interest is water (e.g., recreational waters, drinking water sources, shellfish harvesting waters, biosolids), MRA resources for food safety risk were also consulted in the development of this MRA protocol. This protocol is designed as a flexible tool, amenable to modification where an EPA Office or other user has particular requirements that may not be precisely covered in the text. This MRA protocol is a modular tool box with a broad scope and it is expected that those modular aspects that are relevant to the MRA being conducted can be used as deemed appropriate by the EPA Office conducting the assessment. As this protocol is designed for use by EPA and its contractors, individuals with applicable technical expertise (e.g., microbiologists, risk assessment modelers, public health practitioners), and risk managers it may not meet the needs of other users such as international bodies, foreign governments, other U.S. governmental organizations, and other EPA Offices with different responsibilities or perspectives.¹

This MRA Protocol does not provide discussion and evaluation of state-of-the-art research that is ongoing to support the field of MRA. In some places literature is cited for readers that are interested in exploring topics in MRA's future development. This protocol does not provide instructions for conducting statistical or modeling analysis. Given that a risk assessment team should have the technical expertise to conduct the modeling analysis, this Protocol provides a systematic approach for framing information to be considered, an outline for conducting and documenting risk assessment that is compatible with other well known frameworks, and information to help ensure risk characterization that is helpful and relevant for the decision makers.

This Protocol focuses on MRA as it fits into the more comprehensive framework of Risk Analysis—an overarching term used to describe the interaction of risk assessment, risk management, and risk communication (CAC, 2004; Figure 1). Another complementary framework is the WHO's Water Quality Framework (Figure 2). The WHO Water Quality Framework provides for broad public health-based approaches to allow countries to assess options for meeting public health goals.

Although The National Academies of Science, National Research Council (NRC) developed frameworks for risk assessment, those frameworks have been primarily focused on chemical risks (NRC, 1983, 2008). Microbial interactions with hosts and the environment are different from chemical interactions. This Protocol was developed to accommodate those differences

¹ EPA's National Homeland Security Research Center published a *Compendium of Prior and Current Microbial Risk Assessment Methods for Use as a Basis for the Selection, Development, and Testing of a Preliminary Microbial Risk Assessment Framework*. This literature review includes summaries of 135 studies published between 1994 and 2004, 44 related to exposure assessment (oral, inhalation, and dermal), 31 related to dose-response, and 60 related to risk characterization (EPA, 2007b).

between chemicals and microbes, while maintaining compatibility with the overall NRC frameworks. A summary of factors that make MRA unique from chemical risk assessments is provided in [Appendix B](#).

This Protocol primarily focuses on risk assessment and only addresses risk management and risk communication activities to the extent that they overlap with risk assessment. However, it is important to note that risk assessment is not an effective process unless risk management and risk communication activities are also comprehensively pursued.



Figure 1. Risk Analysis

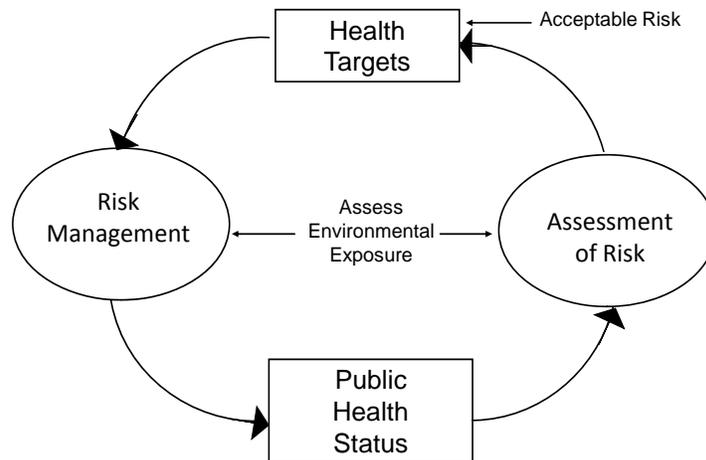


Figure 2. WHO Water Quality Framework
(Source: Adapted from WHO, 2001)

One topic not addressed in this Protocol is how EPA sets priorities for MRA or selects which MRAs to conduct. An example of one approach to priority setting is EPA's drinking water Contaminant Candidate List (CCL) Classification Process.² The U.S. Food and Drug Administration (FDA) also has formal guidelines for determining how to set priorities for initiating microbial risk assessments (FDA, 2002).

Microbial risk assessments can be initiated for a variety of reasons, including but not limited to the following:

- to assess the potential for human risk associated with exposure to a known pathogen;
- to determine critical points for control, such as watershed protection measures;
- to determine specific treatment processes to reduce, remove, or inactivate various pathogens;
- to predict the consequences of various management options for reducing risk;
- to determine appropriate criteria or regulatory levels that will protect individuals and/or populations to a specified risk level or range
- to identify and prioritize research needs; and
- to assist in epidemiological investigations.

Individual risk assessments for specific situations can differ significantly with respect to the questions that are addressed, the information required to address those questions, and the nature of data gaps. [Appendix A](#) presents example flow diagrams of some of the types of risk assessments that are consistent with this MRA Protocol. As illustrated in Appendix A, MRAs may be conducted to characterize the risk associated with a particular combination of a pathogen and route of exposure, "in reverse" to compute a concentration of a specific pathogen that would correspond to a pre-specified level of risk, and/or to evaluate the relative ranking of pathogen/exposure combinations. Examples of each of these approaches are referred to throughout this document.

1.2 Development of the MRA Protocol

This MRA Protocol is comprised of a combination of concepts from numerous published risk assessment frameworks and workshop proceedings. Although many of these frameworks and proceedings were originally developed for other applications such as food safety, there are many principles that also apply to water-related risk assessments. The resources employed to develop this MRA Protocol are briefly summarized below and 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, 2008)
- EPA Office of Water/ILSI RSI *Revised Framework for Microbial Risk Assessment* (ILSI, 2000)
- EPA *Guidelines for Ecological Risk Assessment* (EPA, 1998)

² http://www.epa.gov/ogwdw000/ndwacsum.html#ccl_cp

- EPA *Framework for Cumulative Risk Assessment* (EPA, 2003f)
- EPA Office of the Science Advisor Staff Paper *Risk Assessment Principles and Practices* (EPA, 2004d)
- Codex Alimentarius Commission, *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (CAC, 1999) and *Codex Proposed Draft Principles and Guidelines for the Conduct of Microbial Risk Management* (CAC, 2007)
- Food and Agricultural Organization (FAO) and World Health Organization (WHO), *Microbiological Risk Assessment Series, No.3, Hazard Characterization for Pathogens in Food and Water Guidelines* (FAO/WHO, 2003).
- WHO *Water Quality: Guidelines, Standards and Health, Assessment of risk and risk management for water-related infectious disease* (WHO, 2001)
- MRA Workshops:
 - U.S. EPA Microbiological Risk Assessment Framework Workshop: Tools, Methods, and Approaches (August 2002) (EPA, 2002c)
 - U.S. EPA Microbiological Risk Assessment Framework: Problem Formulation Workshop (July 2003) (EPA, 2003c).

In 1983, in response to a request by the U.S. Congress, the National Academy of Sciences, National Research Council published a risk assessment framework (hereafter referred to as NRC framework) that addressed primarily chemicals (NRC, 1983). It was developed by a committee of volunteer experts drawn from academia, government, and industry that was charged to conduct a study of institutional approaches to risk assessment within the federal government. The NRC committee's report underwent extensive peer review and continues to be widely cited and used in the chemical risk assessment community. The framework has also served as a template for the development of numerous subsequent risk assessments and risk assessment frameworks. In 2008, the NRC Committee on Improving Risk Analysis Approaches Used by the U.S. EPA issued a report that further developed the original 1983 framework by expanding on problem formulation and risk-based decision-making (NRC, 2008). The 2008 NRC framework has the following three phases:

- Phase I: Problem Formulation and Scoping
- Phase II: Planning and Conduct of Risk Assessment
 - Stage 1: Planning
 - Stage 2: Risk Assessment (per the original 1983 NRC framework)
 - Stage 3: Confirmation of Utility
- Phase III: Risk Management

The updated NRC framework also recommends formal provisions for internal and external stakeholder involvement at all stages. In this MRA Protocol, Chapter 2 corresponds to Phase I and Phase II (Stage 1) of the 2008 NRC framework. Chapters 3, 4, and 5 correspond to Phase II (Stage 2) of the 2008 NRC framework. Chapter 5 includes some of the concepts from Phase II (Stage 3) and Phase III of the 2008 NRC framework.

The International Life Sciences Institute's Risk Science Institute (ILSI RSI) and the EPA Office of Water developed a conceptual framework for assessing the risks of human disease following exposure to waterborne pathogens—EPA/ILSI *Framework for Microbial Risk Assessment*

(hereafter called the EPA/ILSI Framework) (ILSI, 1996, 2000). The EPA/ILSI Framework follows the general structure of the EPA *Guidelines for Ecological Risk Assessment* (EPA, 1998). This *Microbial Risk Assessment Protocol* (MRA Protocol) is based on and refines earlier frameworks. Although this MRA Protocol follows the basic structure of the EPA/ILSI Framework, elements of several international frameworks for food and water microbial risk assessment have been integrated into the MRA Protocol to increase the harmonization with international approaches. The EPA/ILSI Framework describes a generic approach to identifying scientific information that should be considered in attempts to quantitatively or qualitatively assess the human health risks associated with exposure to infectious agents in water. The process to develop the EPA/ILSI Framework included three workshops held in 1995, 1996, and 1999; deliberations by a 30-member working group of scientists from academia, industry, and government; and two ILSI quantitative risk assessments (Soller et al., 1999; Teunis and Havelaar, 1999) to test the utility and flexibility of the framework. This MRA Protocol draws on concepts and terminology from all of the above documents, but relies primarily on the EPA/ILSI Framework. The conclusions and recommendations from the EPA/ILSI 1999 workshop are integrated throughout the MRA Protocol where appropriate. Notably, the participants in the 1999 workshop suggested that the framework could be further revised to include a number of additional capabilities. Two specific suggestions that are integrated into this MRA Protocol are the inclusion of specific information on the various types of mathematical models that have been used in MRAs and methods to address time-dependent aspects of infectious disease and immunity (dynamic modeling).

To support the continued enhancement of the EPA/ILSI Framework, EPA convened two workshops, Microbiological Risk Assessment Framework Workshop Tools, Methods, and Approaches in 2002 (hereafter referred to as the tools workshop) (EPA, 2002c) and Microbiological Risk Assessment Framework: Problem Formulation Workshop in 2003 (hereafter referred to as the problem formulation workshop) (EPA, 2003c). The primary purpose of the tools workshop was to identify available analytical tools, methods, and approaches that can improve qualitative and quantitative microbiological risk assessments conducted under the existing EPA/ILSI Framework. Another important objective was to identify major issues that limit the successful application of the existing framework for conducting risk assessments.

The primary purpose of the problem formulation workshop was to further develop the problem formulation stage of the EPA/ILSI Framework. This included elaboration of the roles of risk assessors, risk managers, risk communicators, and stakeholders during the problem formulation stage; guidance for development of conceptual models; and modification of the process diagram (flow chart) for risk assessment. One important conclusion of both workshops was that the EPA/ILSI Framework is applicable to addressing a wide variety of public health issues related to water quality and food safety. In addition to pathogen-specific analysis, risk assessments could be used to evaluate regulatory actions, evaluate groups of pathogens (e.g., viruses), and evaluate surrogates (e.g., turbidity in drinking water). However, the EPA/ILSI Framework does not specifically discuss these types of risk assessments and did not provide examples. The discussion of problem formulation in the problem formulation workshop overlapped with EPA's Science Policy Council and Office of the Science Advisor's definition of Planning and Scoping (EPA, 2000b, 2002b, 2004d). Although the problem formulation workshop participants envisioned problem formulation as encompassing many of the aspects of Planning and Scoping,

for the purpose of this MRA Protocol, problem formulation has been defined as part of the overall Planning and Scoping to be consistent with other EPA risk assessment documents.

A *Thesaurus of Terms Used in Microbiological Risk Assessment* (hereafter referred to as the Thesaurus) has been developed in parallel to this MRA Protocol (EPA, 2007a). The Thesaurus compiles definitions of terms from EPA sources, other U.S. Federal agencies, international guidelines, foreign governments, and several nongovernmental organizations (NGOs) concerned with risk assessment. Definitions in the Thesaurus were evaluated for their potential to cause confusion, such as when the same term has differing definitions depending on its application, or when similar concepts are known by different names in different disciplines. Refer to the Thesaurus for detailed definitions of specific microbial risk concepts.

EPA's Risk Assessment Forum, a standing committee of senior EPA scientists that was established to promote Agency-wide consensus on difficult and controversial risk assessment issues, developed the *Guidelines for Ecological Risk Assessment* (hereafter referred to as Guidelines for ERA) (EPA, 1998). The purpose of these Guidelines is to help improve the quality of ecological risk assessments at EPA while increasing the consistency of assessments among the Agency's program offices and regions. The Guidelines for ERA expand upon and replace the previously published EPA report *Framework for Ecological Risk Assessment* (EPA, 1992), which proposed principles and terminology for the ecological risk assessment process. To develop the Guidelines for ERA, EPA sponsored public and Agency colloquia, developed peer-reviewed ecological assessment case studies, and prepared a set of peer-reviewed issue papers highlighting important principles and approaches. Drafts of the proposed Guidelines for ERA underwent formal external peer review and were reviewed by the Agency's Risk Assessment Forum, by Federal interagency subcommittees of the Committee on Environment and Natural Resources of the White House Office of Science and Technology Policy, and by the Agency's Science Advisory Board (SAB). Although the Guidelines for ERA only apply to non-human receptors and mainly chemical stressors, the Guidelines for ERA were used as a basic outline for the EPA/ILSI Framework, which is the basis of this MRA Protocol; therefore, the Guidelines for ERA also serve as a foundation for this document.

EPA's *Framework for Cumulative Risk Assessment* serves as a foundation for developing future cumulative risk assessment guidelines and it is expected to evolve (EPA, 2003f). "Cumulative risk assessment" means "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors." One key aspect of this definition is that a cumulative risk assessment need not necessarily be quantitative, so long as it meets the other requirements. Cumulative risk involves multiple agents or stressors, which means that the "agents or stressors" can be chemicals, biological agents, physical agents, or an activity that, directly or indirectly, alters or causes the loss of a necessity such as habitat. This definition requires that the risks from multiple agents or stressors be combined. However, this does not necessarily mean that the risks should be "added," but rather that some analysis should be conducted to determine how the risks from the various agents or stressors interact. It also means that an assessment that covers a number of chemicals or other stressors but that merely lists each chemical with a corresponding risk without consideration of the other chemicals present is not an assessment of cumulative risk under this definition.

Community-based cumulative risk assessment is of growing interest to EPA. For example, EPA's Workshop on Research Needs for Community-Based Risk Assessments (October 2007) described community-based risk assessment as follows:³

Community-based risk assessment is a model that addresses the multiple chemical and non-chemical stressors faced by a community, while incorporating a community-based participatory research framework and a transparent process to instill confidence and trust among community members. It has become clear that cumulative risk assessments should include both chemical and non-chemical stressors, exposures from multiple routes, and population factors that differentially affect exposure or toxicity, and in some cases, resiliency to environmental contaminants.

Although the concepts and factors presented in this MRA Protocol could be used to consider microbial risks in the context of community-based cumulative risk assessment, at present there are no examples of cumulative MRA in the literature.

EPA's Office of the Science Advisor's Staff Paper on *Risk Assessment Principles and Practices* reviews EPA's chemical risk assessment practices across the agency (EPA, 2004d). It discusses general risk assessment topics such as "conservatism," default assumptions, Planning and Scoping, uncertainty, variability, and information gaps. Chemical specific concepts are also discussed, such as, maximum tolerated dose, no observable adverse effect level, lowest observable adverse effect level, benchmark dose, toxicity equivalency factor, reasonable maximum exposure, and toxicity reference values. The Staff Paper includes discussion of the historical context of many of these concepts and serves as a useful orientation to EPA chemical risk assessment. The discussion of general topics is also applicable to MRA.

The Codex Alimentarius Commission (Codex) was created by the United Nations/Food and Agricultural Organization (FAO) and World Health Organization (WHO) to develop food standards, guidelines, and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. Since its formation in 1963, Codex has been the internationally acknowledged source for safety guidelines and standards related to international trade in foodstuffs. Codex follows an eight step Elaboration Procedure for drafting, amending, and adopting standards and guidelines. In the final step of the elaboration procedure, documents are adopted by the Commission and sent to the governments of the participating countries for acceptance. In 1999, Codex adopted *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (hereafter referred to as Codex MRA Guidelines) (CAC, 1999). A companion document, *Proposed Draft Principles and Guidelines for the Conduct of Microbial Risk Management*, is at step five of the elaboration procedure (hereafter referred to as Codex draft MRM Guidelines) (CAC, 2007). Although mainly applicable to food safety risk assessment, the Codex MRA Guidelines, and draft MRM Guidelines contain many principles that also apply to water safety risk assessments because important waterborne pathogens can also contaminate foods and food products.

The WHO document *Water Quality: Guidelines, Standards and Health, Assessment of Risk and Risk Management for Water-Related Infectious Disease* (hereafter referred to as WHO WQ

³ http://es.epa.gov/ncer/events/news/2007/10_18_07_calendar.html

Standards) (WHO, 2001), is intended to harmonize⁴ the process of development of guidelines and standards for drinking water, wastewater used in agriculture and aquaculture, and recreational water environments. The harmonized framework was developed through discussions by an international group of experts that included professionals in the fields of drinking water, irrigation, wastewater use, and recreational water, as well as those with expertise in public health, epidemiology, risk assessment/management, economics, risk communication, and the development of guidelines and standards. The series of reviews in the WHO WQ Standards address the principle issues of concern linking water and health to the establishment and implementation of effective, affordable, and efficient guidelines and standards.

The FAO/WHO *Microbiological Risk Assessment Series, No.3, Hazard Characterization for Pathogens in Food and Water Guidelines* (FAO/WHO, 2003) is an overall framework that includes summaries of strengths and limitations of outbreak investigations, surveillance and annual health statistics, volunteer feeding studies, biomarkers, intervention studies, animal studies, *in vitro* studies, and expert elicitation. Elements adapted from the EPA/ILSI Framework are discussed in detail. An outline of information to include in Hazard Characterization is also presented.

1.3 MRA Protocol Framework

This MRA Protocol employs primarily an expanded and enhanced version of the EPA/ILSI Framework for MRA. The overall EPA/ILSI Framework structure is illustrated in Figure 3. For the purposes of this document, the EPA/ILSI structure has been modified in the recognition that MRA practitioners and managers often desire flexibility in the development of MRAs, and that in some cases the NRC chemical risk framework (or another framework) may be preferred. Thus, components of the NRC chemical framework are clearly identified and incorporated in the MRA Protocol described herein.

The fundamental steps in the EPA/ILSI framework are problem formulation, analysis, and risk characterization (Figure 3). Elements of the hazard identification in the 1983 NRC paradigm are included in both the problem formulation and analysis phase of the EPA/ILSI Framework (Figures 3 and 4). The EPA/ILSI analysis phase consists of two major elements—characterization of exposure and characterization of human health effects. The exposure assessment and dose-response assessment from the NRC framework are conducted within the Analysis phase, but are also considered during problem formulation and risk characterization phases. The Risk Characterization phase is also similar between the two frameworks, except that the NRC framework separates risk management from risk assessment conceptually, so that risk management decisions are separate from scientific decisions. The EPA/ILSI paradigm integrates risk management into the risk characterization phase to a greater degree than is suggested by the NRC framework.

Table 1 (ILSI, 2000) lists factors that should be considered during the development and conduct of a microbial risk assessment. These factors are discussed in more depth throughout this MRA Protocol and are presented here as an overview and to serve as a summary checklist. Not all

⁴ In international law, harmonization refers to the process by which different states adopt the same laws (Stone, 2006). In this context it refers to the adoption of similar protocols.

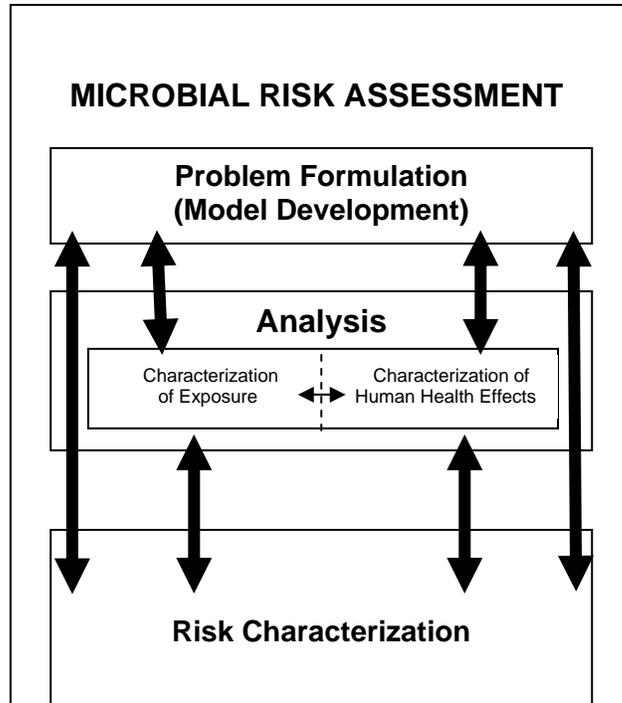


Figure 3. Generalized Framework for Assessing the Risks of Human Disease Following Exposure to Pathogens
 (Source: Adapted from ILSI, 2000)

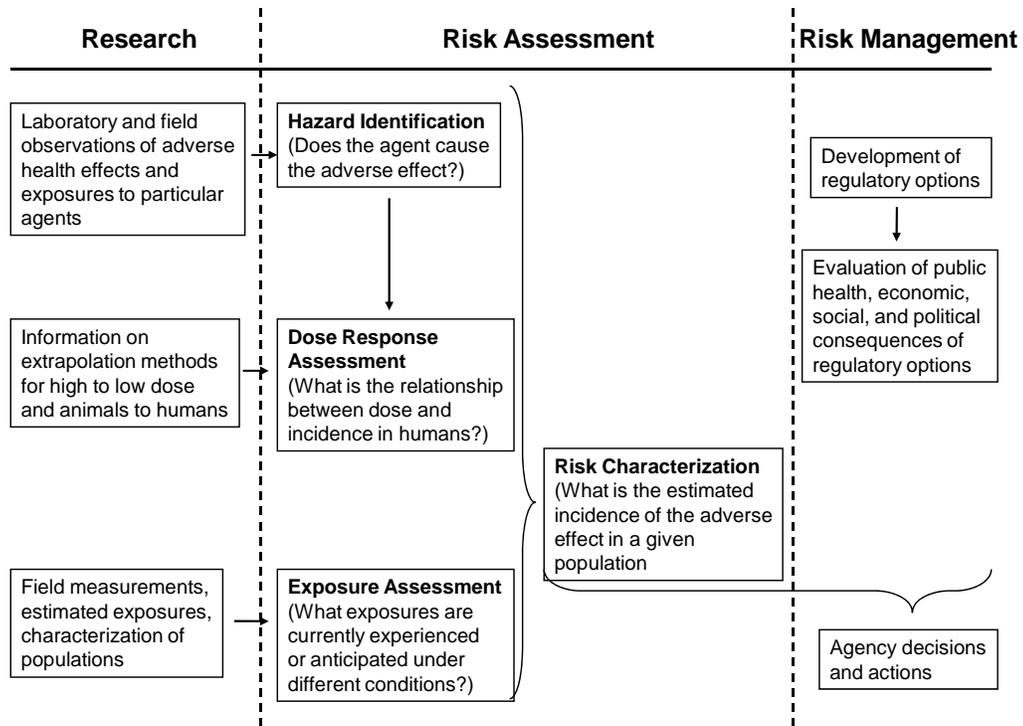


Figure 4. Elements of Risk Assessment and Risk Management
 (Source: Adapted from NRC, 1983)

factors will be appropriate or relevant for all MRAs. However, justification should be provided for excluding a particular factor from an MRA. Note that the factors listed in Table 1 are also referred to as “elements” in this Protocol and may be represented by parameters in an MRA model or may be incorporated into the risk assessment in some other fashion (qualitatively). A brief summary of other risk frameworks that are consistent with this MRA Protocol framework is provided in [Appendix C](#).

The complexity of issues surrounding the design and implementation of a microbial risk assessment requires the use of a flexible tool box approach, in which a variety of readily available tools, methods, resources, and approaches (collectively called tools) are identified for consideration and use at different phases of the assessment. The use of a tool box approach is integral to using this Protocol, although this Protocol does not provide, nor should be inferred to provide, a comprehensive list of tools available for use in microbial risk assessment.

1.4 General MRA Concepts

There are a various concepts and processes that broadly apply to MRAs that are within the scope of this MRA Protocol. The following is a brief overview of these concepts. A more detailed description of these concepts is provided in [Appendix D](#).

- Iterative nature of risk assessment: The risk assessment process is not linear, but flexible and dynamic (ILSI, 2000). During any of the three phases of the microbial risk assessment process—problem formulation, analysis, and risk characterization—the other phases should be revisited and refined as new information and insights become available.
- Transparency, clarity, consistency, and reasonableness (TCCR): Risk assessments must fulfill specific TCCR criteria (EPA, 2000b). The TCCR criteria are summarized as follows:
 - *Transparency*: For risk assessment to be transparent, methods and assumptions should be clearly stated and understandable to the intended audience, whether this consists of informed analysts in the field, risk managers, or the general public.
 - *Clarity* refers to the manner in which the risk assessment is presented, such as writing style and the use of graphic aids.
 - *Consistency* provides a context for the reader, such as whether the conclusions are in harmony with relevant Agency policy, procedural guidance, and scientific rationales, and if not, how and why the conclusions differ.
 - The *Reasonableness* criteria address the extent to which professional judgments and assumptions are well founded, as confirmed by expert peer review. Risk characterizations should be consistent in general format, but recognize the unique characteristics of each specific situation.
- Data quality: Data used in an EPA risk assessment must be consistent with EPA’s *Information Quality Guidelines* (EPA, 2002a). These Guidelines build upon ongoing efforts to improve the quality of the data and analyses that support EPA’s various policy and regulatory decisions and programs. They create a mechanism that enables the public to seek and obtain, as appropriate, correction of information disseminated by EPA.
- Data representation: In assessing risk associated with infectious disease hazard

Table 1. Elements of Microbial Risk Assessment (Source: Adapted from ILSI, 2000)

| Executive Summary | | Elements |
|---|---------------------------|---|
| Problem Formulation Chapter | Pathogen Characterization | Virulence and pathogenicity of microorganism |
| | | Pathologic characteristics/disease caused |
| | | Survival and multiplication |
| | | Resistance to control or treatment processes |
| | | Host specificity |
| | | Infection mechanisms/route of infection/portals of entry |
| | | Potential for secondary spread |
| | | Taxonomy/strain variation |
| | | Ecology and epidemiological triad |
| | Host Characterization | Age |
| | | Immune status |
| | | Concurrent illness/medical treatment |
| | | Genetic background |
| | | Pregnancy |
| Exposure Chapter | Pathogen Occurrence | Nutritional status |
| | | Social/behavioral traits |
| | | Temporal distribution/frequency |
| | | Concentration in environmental media |
| | | Spatial distribution (clumping, aggregation, particles, clustering) |
| | | Niche (ecology, non-human reservoirs) |
| | | Survival, persistence, amplification |
| | | Seasonality |
| | Exposure Analysis | Meteorological and climatic events |
| | | Presence of treatment or control processes |
| | | Identification of media (water and shellfish for Ambient Water Quality Criteria [AWQC]) |
| | | Units of exposure |
| | | Routes of exposure |
| | | Size of exposed population |
| Health Effects Chapter | Health Effects | Demographics of exposed population |
| | | Spatial and temporal nature of exposure (whether single or multiple exposures) |
| | | Behavior of exposed population |
| | | Duration of illness |
| | | Severity of illness |
| | Dose-response | Infectivity |
| | | Morbidity, mortality, sequelae of illness |
| | | Extent or amount of secondary transmission |
| | | Human and/or animal dose-response data |
| | | Outbreak or intervention data |
| | | Route of exposure or administration |
| | | Source and preparation of challenge material or inoculum |
| | | Organism type and strain (including virulence factors or other measures of pathogenicity) |
| | | Characteristics of the exposed population (age, immune status, etc.) |
| Risk Characterization | Risk Characterization | Duration and multiplicity of exposure |
| | | Infection or disease endpoint (e.g., pathogen shedding, serological response, symptoms) |
| | | Statistical model(s) to analyze or quantify dose-response relationships |
| | | Evaluate health consequences of exposure scenario (risk description [event]) |
| | | Characterize uncertainty/variability/confidence in estimates |
| | | Conduct sensitivity analysis (evaluate most important variables and information needs) |
| | | Address items in problem formulation |
| | | Summarize key issues and conclusions |
| | | Ensure transparency, clarity, consistency, and reasonableness (TCCR) |
| | | Summarize assumptions including explanation of use of default values and methods |
| Describe overall strengths and limitations | | |
| Discuss how a specific risk and its context compares with similar risks | | |

exposures, it is usually necessary to estimate a number of parameters (quantities) in the risk models (equations) that yield numerical estimates of the probability of infection. Depending on the data quality, different statistical measures (mean, median, specific percentile values) of these parameters may be appropriate.

- **Data variability and uncertainty:** Uncertainty and variability can impact the quality and interpretation of MRA model results. Understanding, accounting for, and communicating the impacts of these factors is critical in an MRA. The EPA *Exposure Factors Handbook* (EPA, 1997a, 2000b) indicates that uncertainty represents a lack of knowledge about factors affecting exposure or risk, whereas variability arises from true heterogeneity across people, places, or time.
- **Model validation:** Model validation and verification in risk assessment are general terms that are sometimes used to refer to rigorous data driven evaluation of models. However, these terms are often used interchangeably to refer to a less rigorous “reality check” that may have poorly defined validation criteria. Because validation implies different criteria in different situations, any discussion of validation should refer to how the validation was performed so that readers may properly understand the degree of rigor that the validation effort entailed. For example, one method that has been used to validate risk assessment findings is to compare the outputs to epidemiological data to determine whether the risk estimates are consistent with reality.
- **Risk assessment team:** Risk assessment teams are multidisciplinary and may include individuals with expertise in diverse disciplines including economics; law; engineering; the sciences (such as microbiology, epidemiology, toxicology, chemistry, and medicine); statistics; mathematics; software programming; website design; and technical writing. Although individuals may have overlapping roles, it is important that conflicts of interest between risk assessors and risk managers be avoided to maintain the scientific integrity of the process and stakeholder confidence. Risk assessment and risk management roles for risk assessment team members should be clearly defined.
- **Stakeholders:** The term “stakeholders” refers to people and organizations that can shape the process or will be (or perceive themselves to be) impacted by the risk assessment. Stakeholders should be involved in the Planning and Scoping in a meaningful way. At a minimum, they should be informed about the risk assessment problem, how it is to be addressed, and have an opportunity to provide comments. When stakeholders are directly affected by the proposed assessment, stakeholder comments should be sought to help team members better understand and define the problem. Stakeholders should also be informed periodically of any changes in the problem formulation.
- **Peer review:** The role of peer review is to enhance the quality and credibility of EPA decisions by ensuring that the scientific and technical work products underlying these decisions receive appropriate levels of peer review by independent scientific and technical experts. EPA’s *Peer Review Handbook* provides guidance on conduct of peer review (EPA, 2000a).

There are some long-term goals in the microbial risk assessment field that cannot yet be adequately addressed by tools and methods that are currently available. As the field of microbiological risk assessment advances, this MRA Protocol may be expanded or modified to include new tools once they have been tested and gain general acceptance in the discipline. In addition, some goals have ambitious data requirements that cannot be adequately addressed at

this time. Development of methods to advance MRA capabilities is a general goal of the MRA field. Some examples of possible long-term development goals for microbial risk assessment are presented in [Appendix E](#).

2. Planning & Scoping and Problem Formulation

During Planning and Scoping the purpose of the risk assessment is defined through a dialogue between risk assessors, risk managers, risk communicators, and stakeholders. To be consistent with EPA's Science Policy Council and Office of the Science Advisor's documents on human health risk assessment, Planning and Scoping is considered as the broad set of activities necessary for successfully initiating a risk assessment. The overall Planning and Scoping considers the risk assessment within the context of overall agency resources (EPA, 2000b, 2002b, 2004d).

Problem formulation falls within Planning and Scoping and may continue iteratively throughout the conduct of the risk assessment process (EPA, 2000b, 2002b, 2004d). The purpose of the problem formulation process is to develop the scope of the risk assessment, taking into account management needs, Agency risk assessment policies, risk assessment tool availability, and data constraints.⁵ Problem formulation can provide a written record of the justification for the decisions regarding the scope, goals, and necessary documentation of the risk assessment.

For human health risk assessment, EPA considers Planning and Scoping steps (based on the *Framework for Cumulative Risk Assessment*) (EPA, 2004d) to be as follows:⁶

- defining the purpose of the assessment;
- defining the scope of analysis and products needed;
- agreeing on participants, roles and responsibilities;
- agreeing on depth of assessment and analytical approach;
- agreement on resources available and schedule;
- problem formulation;
- developing the conceptual model; and
- constructing the analysis plan.

EPA's Office of the Science Advisor summarizes problem formulation in ecological risk assessment in the following manner (EPA, 2004d):

Problem formulation, which follows...planning discussions, provides a foundation upon which the entire risk assessment depends. Successful completion of problem formulation depends on the quality of three products: assessment endpoints, conceptual models, and an analysis plan. Since problem formulation is an interactive, nonlinear process, substantial reevaluation is expected to occur during the development of all problem formulation products.

Both human health and ecological risk assessment refer to an analysis plan, which is defined as follows (EPA, 2004d):

⁵ Codex refers to this stage as "risk profile."

⁶ This MRA Protocol defines problem formulation a bit more broadly than the *Framework for Cumulative Risk Assessment* (EPA, 2004d). For example, purpose, scope, depth of assessment, conceptual plan, and analysis plan are all considered part of problem formulation.

The analysis plan “describes how hypotheses about the relationships among the sources, stressors, exposure conditions, populations, and adverse effects/endpoints presented in the conceptual model and narrative will be considered during the risk analysis phase of the assessment. The plan includes a rationale for which relationships are to be addressed and which methods and models will be used and discusses data gaps and uncertainties. The plan may also compare the level of confidence needed for the management decision with the confidence levels expected from alternative analyses in order to determine data needs and evaluate which analytical approach is best.”

It is not necessary to rigidly delineate various activities as part of Planning and Scoping versus problem formulation. It is sufficient to understand that problem formulation includes discussion of scientific and science policy choices related to the conduct of risk assessment while Planning and Scoping includes problem formulation and the operational, logistical, and budgetary planning necessary to successfully conduct the risk assessment.

2.1 Introduction to Problem Formulation within Planning and Scoping

Tasks for problem formulation include describing specific risk management questions, determining data and resource needs, performing preliminary exposure and health effects assessments, developing a conceptual model, and defining key assumptions. Forming an operational plan for conducting the risk assessment should also be accomplished during Planning and Scoping. If it is determined that a full risk assessment is not needed or is infeasible, information gleaned from the problem formulation stage could be used as a qualitative risk assessment or even a semi-quantitative risk assessment, and the process may, in fact, stop after the problem formulation stage. This stepwise approach can be a means of prioritizing resources and defining the scope of the overall risk assessment and to determine whether sufficient information is available to conduct a comprehensive quantitative risk assessment, if in fact, the risk management questions require a comprehensive assessment. Wooldridge and Schaffner (2008) provide guidance on qualitative risk assessment.

Identification of the nature of required inputs and outputs is necessary during problem formulation. Two general risk assessment approaches are consistent with this MRA Protocol. In the first approach, hazard occurrence, exposure assessment, and dose-response assessment are combined to arrive at an estimated risk level. This first approach would be used, for example, to characterize the risk associated with a specific pathogen through specific route of exposure. In the second approach, which is often used for regulatory purposes, dose-response assessment (or exposure-response in the case of epidemiological linkage of hazard to health effect), exposure assessment, and a target risk level or risk range⁷ are combined to determine a hazard occurrence level or concentration that would provide a pre-specified level of public health protection. In the first approach, the estimated risk (e.g., daily or annual risk of infection or illness) is the output; in the second approach, the hazard level (concentration of pathogen/indicator in water corresponding to the target risk) for a given exposure scenario is the output. There are also other types of risk assessments that may be consistent with this MRA Protocol, including the following:

⁷ “Target risk range” is similar to “appropriate level of protection” (ALOP), which is used in the World Trade Organization “Agreement on the application of sanitary and phytosanitary measures” (SPS agreement) and the Codex MRM Guidelines (CAC, 2005).

- product/pathogen pathway analysis—used mainly for microbial risks in a specific food; the risk assessment models the temporal/spatial pathway a product follows through production to consumption);
- risk ranking—ranks risks of same pathogen from multiple sources, or ranks risks of multiple pathogens from one source (For example see FDA-USDA *Listeria* risk assessment (FDA/USDA 2003));
- risk/risk analysis—compares risks between different scenarios, usually management options); and
- geographical introduction analysis—used to estimate risk of introduction of disease agents through food animals or animal products (e.g., intentionally as in bioterrorism or unintentionally) to a region; for example, the risk of bovine spongiform encephalopathy (“mad-cow disease”) occurring in U.S. herds due to importation of livestock from other countries.

All of these types of risk assessments may have different types of outputs and require different inputs. The information presented in this MRA Protocol should be evaluated within the context of the scope of a given risk assessment.

The WHO WQ Guidelines (WHO, 2001) include methods for risk assessments that have health targets, water quality targets, or a performance target that includes engineering technology (including technological approaches for small communities). In this context MRA can be used to (1) provide estimates of the burden of disease, (2) establish norms and standards such as water quality, (3) assess the safety of a system against performance standards, and (4) assess health impacts. The WHO WQ Guidelines methodology is similar to the Codex microbiological risk management (MRM) approach (CAC, 2007) in that it relates public health goals to “food safety objectives,” “performance objectives,” “performance criteria,” and “microbiological criteria.”

During the problem formulation stage, the above concepts can be discussed in the text of one or more of the suggested problem formulation components. These components, which are discussed below, include the statement of concern, statement of purpose, questions, and conceptual model narrative. For example, a risk assessment that estimates the burden of disease could compare water treatment processes, which is a technology-based performance perspective.

The problem formulation process diagram is shown in Figure 5. Note that this diagram does not include specifics about what questions could be asked or how the conceptual model should be built. However, it does show the types of information that should be collected to determine the feasibility of conducting a quantitative microbial risk assessment. The diagram is roughly chronological. Initially, a concern or set of concerns

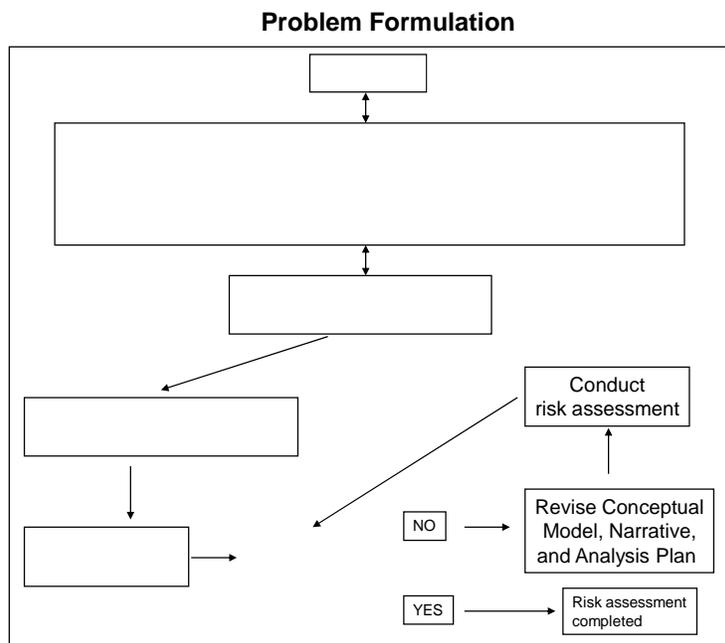


Figure 5. Enhanced Problem Formulation Process Diagram
(Source: Adapted from EPA, 2003c)

is identified. Those concerns can come to the attention of the Agency through various routes. The statement of concern, statement of purpose, and questions to be considered evolve throughout the problem formulation stage. These can be integrated into a risk assessment “charge.” Risk managers are responsible for ensuring that appropriate problem formulation documentation is developed so that it is sufficient for the particular problem at hand. With initial information regarding the scope and questions for the risk assessment, risk assessors determine the feasibility of carrying out those plans given the available data, risk assessment tools, and time and resources. A concise conceptual model, narrative, and analysis plan are developed. A screening-level risk assessment may first be performed to determine if the risk assessment questions can be addressed without an extensive formal quantitative risk assessment. In some cases, a screening level risk assessment may be adequate for decision making. If a formal quantitative risk assessment is desired and feasible, a more detailed conceptual model/narrative and analysis plan are developed. The problem formulation documentation can be used to assist risk managers with policy decisions that are needed to define the scope of the risk assessment. For example, risk assessors can outline options for risk managers to consider. Risk assessment is iterative by nature, and thus aspects considered during the problem formulation may need to be revisited multiple times as new information and/or data become available. During problem formulation, the risk assessment options that are considered, the options that are chosen, and the justification for those decisions, should be carefully tracked.

It should also be noted that risk assessments can be developed in phases; as indicated previously, a screening level risk assessment may be the initial step that later leads to an enhanced fully quantitative risk assessment. The complexity of the risk assessment may be incrementally increased by addition of new models or parameters, or by more rigorously characterizing parameter values (e.g., from point estimate values to a statistical distribution). In many cases,

sensitivity analysis can guide prioritization regarding further data gathering or refinement of parameter estimates. The iterative nature of the problem formulation process should allow for further definition and refinement of possible phases of the risk assessment. If multiple versions of the risk assessment are run as a result of this iterative process, the choices for each version (also referred to as phase) of the risk assessment should be tracked and documented.

2.2 Documenting the Problem Formulation and Planning and Scoping

The purpose of the problem formulation and planning and scoping process is to develop the scope of the risk assessment, taking into account management needs, Agency risk assessment policies, risk assessment tool availability, data constraints, and overall Agency resources. A valuable aspect of this process is documenting the problem formulation development. The form of this documentation can vary depending on the needs of the EPA Office conducting the assessment. The range of acceptable forms for this documentation ranges from a formal and stand-alone problem formulation document to internal notes kept by the project (or work assignment) manager for the Office conducting the assessment. The value of documenting the problem formulation process is that it provides a written record of the justification for the decisions regarding the scope, goals, and necessary documentation of the risk assessment. During the problem formulation process, the purpose of the risk assessment is defined through a dialogue between risk assessors, risk managers, risk communicators, and if appropriate, stakeholders.

During development of the problem formulation documentation, it should be kept in mind that the concepts and, in many cases, the language therein could be used in the final risk assessment document. Depending on the form of the problem formulation documentation, the statement of concern and the statement of purpose can be included as part of an executive summary of the risk assessment document. The scope, questions to be addressed, conceptual model, and data not included can be used in the problem formulation chapter of the risk assessment document. Other planning and scoping documentation can be summarized in the problem formulation chapter, a planning and scoping chapter or, if desired, attached as an appendix. The tools, data inventory, summary of assumptions, and discussion of recommended factors are used as appropriate in the exposure and human health chapters of the risk assessment document. The summary of assumptions is reiterated in the risk characterization chapter, which also includes the discussions of variability, uncertainty, and identified gaps.

An example outline that could be used for a problem formulation/planning and scoping document is presented below, the components of which are summarized in the following sections.

- 1) Statement of Concern**
- 2) Statement of Purpose and Objectives**
- 3) History and Context within the Agency**
- 4) Scope and Risk Range**
 - (a) Define the hazard (pathogen strain[s], indicator, other)
 - (b) Define which populations will be included in the risk assessment model (explicitly and implicitly)

- (c) Define health outcomes or endpoints (including how the health outcome is measured)
 - (d) Define what unit of exposure and route of concern is relevant (time-span of exposure) and why
 - (e) Define level of protection (target risk range) and basis for the target levels (not applicable if risk estimate is output)
 - (f) List specific scenarios the risk managers would like to model (varying the inputs)
- 5) Questions to be Addressed**
- (a) Questions the risk assessment should be able to answer
 - (b) Questions for the risk managers
- 6) Conceptual Model (Figure)**
- (a) Top Tier: Flow Chart (could be an influence diagram) of how risk is thought to occur in the big picture (limit scope to area covered in risk assessment)
 - (b) Conceptual Model Sub-Tiers: Flow chart of more detailed nodes that will be modeled in the risk assessment (note, the level of detail in the conceptual model diagram should match the level of detail that will be addressed in the risk assessment)
- 7) Conceptual Model Narrative**
- The following sections should be included in the conceptual model narrative:
- (a) **Tools** (software, methods for dealing with uncertainty, dose-response models, exposure models, outline validation approach)
 - (b) **Data inventory** (estimate quality and quantity, include sources for model validation); summarize literature search (present **literature search strategy** in appendix)
 - (c) **Summary of assumptions** (including default value assumptions)
 - (d) **Sources of variability**
 - (e) **Sources of uncertainty**
 - (f) **Factors and data not included and justification**
 - (g) **Identified gaps in the knowledgebase**
 - (h) **Relevant environmental sampling strategies and analysis methods**
 - (i) **Intervention action options** and places where interventions can take place (if within the scope of the risk assessment)
- 8) Summary of other Planning and Scoping Activities**
- Operational Plan (Logistics)**
- The operational plan can include a process diagram and graphic or table timeline. The below items may be covered in a work plan or other management documents. Documents that contain the information below may be referred to in the problem formulation documentation, but the information does not need to be duplicated. The following information should be addressed during Planning and Scoping:
- (a) Brief description and **list of anticipated deliverables**
 - (b) **Milestones** that trigger team meetings and goals of each meeting
 - (c) **Assign specific team members to tasks** (e.g., modeling and writing tasks)
 - (d) **Prioritize resources**
 - (e) **Timeline** should include meetings, deliverables, appropriate placeholders for other interactions (with risk communicators, stakeholders, upper management), milestones for performing quality audits

- (f) **Plan for informal and formal peer review** (e.g., use peer review subcontractor, Science Advisory Board, Risk Assessment Forum; see also Appendix D)

For comparison, the risk profile approach developed by Codex for microbiological food risk is presented in Text Box 1. Note that in the Codex paradigm, the risk profile is similar to and serves the same purpose as problem formulation described in this MRA Protocol.

Text Box 1. Microbiological Risk Profile for Food

(Source: Adapted from CAC, 2007)

A risk profile should present, to the extent possible, information on the following.

1. Hazard-food commodity combination(s) of concern:
 - Hazard(s) of concern
 - Description of the food or food product and/or condition of its use with which problems (foodborne illness, trade restrictions) due to this hazard have been associated
 - Occurrence of the hazard in the food chain
2. Description of the public health problem:
 - Description of the hazard including key attributes that are the focus of its public health impact (e.g., virulence characteristics, thermal resistance, antimicrobial resistance)
 - Characteristics of the disease, including:
 - Susceptible populations
 - Annual incidence rate in humans including, if possible, any differences between age and sex
 - Outcome of exposure
 - Severity of clinical manifestations (e.g., case-fatality rate, rate of hospitalization)
 - Nature and frequency of long-term complications
 - Availability and nature of treatment
 - Percentage of annual cases attributable to foodborne transmission
 - Epidemiology of foodborne disease
 - Etiology of foodborne diseases
 - Characteristics of the foods implicated
 - Food use and handling that influences transmission of the hazard
 - Frequency and characteristics of foodborne sporadic cases
 - Epidemiological data from outbreak investigations
 - Regional, seasonal, and ethnic differences in the incidence of foodborne illness due to the hazard
 - Economic impact or burden of the disease if readily available
 - Medical, hospital costs
 - Working days lost due to illness, etc.

Text Box 1. Continued

3. Food production, processing, distribution, and consumption:
 - Characteristics of the commodity (commodities) that are involved and that may impact on risk management
 - Description of the farm to table continuum including factors which may impact the microbiological safety of the commodity (i.e., primary production, processing, transport, storage, consumer handling practices)
 - What is currently known about the risk, how it arises with respect to the commodity's production, processing, transport and consumer handling practices, and who it affects
 - Summary of the extent and effectiveness of current risk management practices including food safety production/processing control measures, educational programs, and public health intervention programs (e.g., vaccines)
 - Identification of additional risk mitigation strategies that could be used to control the hazard
4. Other risk profile elements:
 - The extent of international trade of the food commodity
 - Existence of regional/international trade agreements and how they may affect the public health impact with respect to the specific hazard/commodity combination(s)
 - Public perceptions of the problem and the risk
 - Potential public health and economic consequences of establishing Codex Microbial Risk Management (MRM) guidance document
5. Risk assessment needs and questions for the risk assessors:
 - Initial assessments of the need and benefits to be gained from requesting an MRA, and the feasibility that such an assessment could be accomplished within the required time frame
 - If a risk assessment is identified as being needed, recommended questions that should be posed to the risk assessor
6. Available information and major knowledge gaps provide, to the extent possible, information on the following:
 - Existing national MRAs on the hazard/commodity combination(s)
 - Other relevant scientific knowledge and data that would facilitate MRM activities including, if warranted, the conduct of an MRA
 - Existing Codex MRM guidance documents (including existing Codes of Hygienic Practice and/or Codes of Practice)
 - International and/or national governmental and/or industry codes of hygienic practice and related information (e.g., microbiological criteria) that could be considered in developing a Codex MRM guidance document
 - Sources (organizations, individual) of information and scientific expertise that could be used in developing Codex MRM guidance document
 - Areas where major absences of information exist that could hamper MRM activities including, if warranted, the conduct of an MRA

2.2.1 Statement of Concern

A concise statement of concern should be developed during problem formulation to convey, in simple terms, what hazard is being addressed and how it is thought to relate to human health for an exposure scenario.

2.2.2 Statement of Purpose and Objectives

The purpose and/or objectives of the risk assessment should be stated in a concise paragraph.

Example language for risk assessments performed for the purpose of derivation of AWQC for a specific pathogen is provided below. Note, the designated use and the national scope might be different in other cases.

This risk assessment is being performed as an essential component for deriving Ambient Water Quality Criteria (AWQC) for *[pathogen or indicator name]* under §304(a) of the Clean Water Act (CWA). These will be nationally recommended AWQC for the protection of the *[insert designated use]* designated use. It should not be implied that the AWQC will be protective of other designated uses, such as *[insert designated uses that are excluded]*. As with other §304(a) AWQC, the AWQC for *[pathogen or indicator name]* are recommended for adoption by states to be used for total maximum daily load (TMDL) determination. States also use AWQC to help assess whether water bodies are threatened or impaired (§305b or §303d CWA) for the specified designated use.

2.2.3 History and Context within the Agency

Previous risk assessments addressing the same or similar hazards should be summarized to provide context for the current risk assessment. In particular, if previous EPA risk assessments have been conducted, then the relationship between the current and previous risk assessments should be summarized. Relevant information for presenting updated MRAs may include new mandates, policy developments, technical advancements, risk assessment method and tool advancements, and new or enhanced data sets.

2.2.4 Scope

The scope section of problem formulation outlines the scenarios that the risk assessment will address. It is often most helpful to list several options for answering the questions listed below. Then, managers and assessors can engage in a dialog to determine which options will be used. The scope should summarize the following:

1. Which infectious disease hazard is being addressed (pathogen strain[s] or indicator[s])? Define the hazard.
2. Which human populations will be included in the risk assessment (e.g., general population or subpopulations, or geographically defined populations)? Describe which populations are explicitly included in the risk assessment model, which will be accounted for implicitly, and which populations may be excluded by the risk assessment model (e.g., most extreme behaviors).
3. What health outcomes or endpoints are addressed by the risk assessment, including how the health outcome is measured? Clearly defining the health endpoint is important for transparency and also focuses the scope of the risk assessment (e.g., infection, disease symptom/s, mortality).
4. What unit and routes of exposure are relevant and why (time-span of exposure)?
5. For risk assessments designed to derive criteria to set “safe” levels of microorganisms, what level of protection (target risk or risk range) will be provided by the criteria, and what is the technical or policy justification for those criteria?

6. What specific exposure scenarios will be modeled? List specific scenarios the risk managers would like to model (varying the inputs), including desired spatial and temporal features.

2.2.5 Risk Ranges

Currently, EPA does not have an Agency-wide policy for defining acceptable levels of health-based risk associated with pathogenic microorganisms. In fact, there are various regulatory requirements that influence the degree to which MRAs conducted within the Agency are driven by risk ranges. For example, historically accepted risk ranges for illnesses due to waterborne pathogens in recreational waters were used to derive the 1986 AWQC for Bacteria. The accepted risk level is 8/1000 for fresh recreational water (8 acute gastrointestinal illnesses (AGI) in 1000 persons exposed) and 19/1000 for marine waters (EPA, 1986a,b). In contrast, current policy for drinking water standards is to characterize the degree of protectiveness without having targets or acceptable risk. In this approach the protective ranges have been influenced mainly from feasibility of measurement and application of control technology, taking costs into consideration. Furthermore, it should be clear that semi-quantitative or qualitative MRAs may be necessary under some conditions and that these assessments may still be meaningful for risk management decisions. For example, it may be possible to evaluate the relative degree of protection from fecal contamination in drinking water sources without quantitatively characterizing the risk associated with a specific health endpoint.

Although acceptable risk and target risk are not necessarily always the same, they are both numeric values that are determined through science-policy decisions. There may be an expectation among some stakeholders that a certain target risk range is acceptable. However, given that different stakeholders may have different ideas about what is acceptable and what is not, it may be misleading to label a risk range “acceptable.” Risk ranges are values that can be estimated empirically from data. However, there may not be clear or convincing information to determine if historically accepted risk ranges are considered acceptable to current stakeholders or not. When risk ranges are used as a driving force or target for MRA conduct within OW, the risk range is defined along four dimensions, as described below:

1. Risk range is for a specified population (population can be defined in a variety of ways, such as “general,” highly exposed, or highly susceptible).
2. Risk range is associated with a defined health endpoint.
3. Risk range covers a defined time span of exposure.
4. Risk range may also be linked to a specific exposure scenario. For example, AWQC are based on a designated use, which indicates exposure routes.

Several representative examples of risk ranges employed currently by EPA are presented in Text Box 2.

Text Box 2. Information Used to Establish Risk Ranges and Representative Examples of Risk Ranges Currently Employed by EPA

| Pollutant | Specified Population | Health Endpoint | Time Span ^a | Exposure (Designated Use) |
|---|--------------------------|-----------------|--------------------------|------------------------------------|
| Carcinogen | General | Cancer | Lifetime | All water uses |
| Carcinogen | Highly exposed subgroups | Cancer | Lifetime | All water uses |
| Indicator bacteria (average concentrations) | General | AGI | Per recreational event | Freshwater or marine recreation |
| <i>Cryptosporidium</i> (average concentrations) | General | Infection | Annual risk of infection | Treated drinking water consumption |

^a Time span for exposure should not be confused with time spans relevant for monitoring protocols.

Specific EPA examples:

- EPA’s surface water program has derived AWQC for chemical carcinogens that generally correspond to lifetime excess cancer risk level of 10⁻⁶ (1 cancer in a million exposed individuals); however, AWQC may correspond to a range from 10⁻⁷ (1 cancer in 10,000,000 exposed individuals) to 10⁻⁵ (1 cancer in 100,000 exposed individuals) (EPA, 2000c).
- Under EPA’s drinking water program (Safe Drinking Water Act [SDWA]), the Long Term 2 Enhanced Surface Water Treatment Rule (LT2) established source water treatment bins for *Cryptosporidium*. The level of public health protection that is provided by LT2 was driven by a concern for misclassification of binning and cost feasibility for the number of samples that could be monitored. Thus, the ranges of public health protection provided by LT2 are an outcome of this risk management approach rather than a pre-specified target risk range (EPA, 2003a,b, 2006a).

Note that in these examples the health outcomes are different (cancer, AGI illness, and infection). This is an important distinction because infection does not always result in illness.

2.2.6 Questions to be Addressed

Microbial risk assessments should be scientifically defensible and relevant to regulatory and public health concerns. Therefore, the risk assessment should be framed within the context of Agency policy. The nature and the specifics of the risk management options that need to be evaluated should be developed during problem formulation, so that the risk assessment design can address any questions that the risk managers want answered. The questions are important for transparency and communication between risk managers and risk assessors. Text Box 3 illustrates this point with three examples of questions that risk managers could ask. There may be two types of questions, (1) questions the risk assessment should be able to answer, and (2) questions that the risk assessors need to have answered by the risk managers for appropriate design of the risk assessment. The second point highlights the need for iterative interaction between risk assessors and risk managers.

Text Box 3. Examples of Risk Management Questions that Could Motivate an MRA Investigation

- What effects have broad-based health programs or specific actions (e.g., health education about disinfection) had on (1) the risk of a specific disease (e.g., cryptosporidiosis) and (2) gastroenteritis (AGI) risks among children?
- Which pathogens (or indicators/surrogates) are associated with human health risks from a specified exposure scenario (e.g., freshwater recreation activities)?
- Are there reduced risks to public health associated with implementation of specific water treatment technologies?

2.2.7 Conceptual Model

A conceptual model is a graphical representation of the real-world scenario that is being addressed in a given risk assessment (EPA, 2002b). There should also be an accompanying narrative that explains the conceptual model. The scope of the risk assessment should be consistent with the conceptual model.

The EPA problem formulation workshop (EPA, 2003c) recommended that multi-tiered conceptual models be constructed. The first (top) tier of the model should be relatively simple, representing only the major components of the assessment. Sub-tier conceptual models can build in more complexity and may require several iterations. The conceptual model should reflect the uniqueness of the situation that is to be addressed. In some cases, a visual diagram that represents how the risk assessment is assembled in the actual software code may serve as a useful sub-tier conceptual model. Although useful for documenting the technical details of the risk assessment, this type of software code map may not clearly communicate the concepts, so should not be solely relied upon as a conceptual model. Collectively, the conceptual model(s) and its narrative should do the following:

- illustrate the risk hypothesis (e.g., provide a flow chart of how risk is thought to occur, within the context of the risk assessment scope);
- outline the tools needed to assess the risk (statistical and other models);
- identify available databases that are needed;
- identify default assumptions;
- show what the risk assessment will or will not be able to do, including whether the assessment is quantitative or qualitative;
- summarize data gaps and quality of data;
- consider the interactions between agent, host, and environment when evaluating risk;
- define key uncertainties;
- identify nodes in the risk assessment, including a brief description of the node and what can happen at the node;⁸ and
- identify intervention actions and places where they can take place.

⁸ For example, rainfall, sunlight, and wind speed/direction could each be separate nodes in a microbial risk assessment. Relevance of nodes can be evaluated by performing sensitivity analysis.

Some of the benefits of developing a conceptual model include the following (from EPA, 1998):

- The process of creating a conceptual model is a powerful learning tool to inform the conduct of the MRA.
- Conceptual models are easily modified as knowledge increases.
- Together with their narrative description, conceptual models highlight what is known and not known and can be used to plan future work.
- Conceptual models can be a powerful communication tool. They provide an explicit expression of the assumptions and understanding of a system for others to evaluate.
- Conceptual models provide a framework for prediction and are the template for generating more risk hypotheses.

It is important that the conceptual model remain free of risk assessment process elements because trying to reflect the risk assessment process in the conceptual model weakens the conceptual model's ability to represent real-world scenarios. Therefore, the risk assessment processes (allocation of Agency resources and deliverable schedule) should be represented separately from the conceptual model (see analysis plan). Because diagrams can be interpreted in different ways by different people, it is essential that a narrative accompany the conceptual model diagram. Details about elements should be included in the text and not clutter the diagram.

Although the concepts of problem formulation and risk assessment analysis can be separated and discussed in a linear manner, the actual process of problem formulation and analysis development is an iterative process. The problem formulation stage should be revisited as the risk assessment takes shape. Defining the scope of the risk assessment and choosing an appropriate model may require several iterations, especially if the risk assessment addresses risks or scenarios that have not been modeled previously.

During problem formulation and developing the first drafts of the risk assessment, it should be possible to determine how complex the risk assessment model needs to be to address the questions posed by risk managers. In some cases where the risk assessment questions are simple and limited in scope, a qualitative risk assessment or a simple risk assessment model may be adequate, even when robust data sets are available. As a general guideline, models should only be as complex as they need to be to address the specific risk management questions. A useful model can help the Agency allocate resources and develop a research agenda as well as provide transparency. A simplified model may help the public better understand the process and should thus accompany a very complex model. Within this context, the conceptual model can also be used by the Agency to consider resource allocation and to develop a research agenda.

Figure 6 presents an example overview or top-tier conceptual model for a risk assessment. In this example, the model summarizes how waterborne risk from *Cryptosporidium* is thought to occur. The conceptual model diagram is a visual representation of the risk hypotheses. The risk hypotheses are the proposed answers to risk assessment questions about how exposure occurs and what endpoints are important for the human health hazard. It should be noted that risk hypotheses are not equivalent to statistical testing of null and alternative hypotheses. However, predictions generated from risk hypotheses can be tested in a variety of ways, including standard statistical approaches (EPA, 1998).

2.2.8 Components in the Conceptual Model Narrative

The following sections should be included in the conceptual model narrative.

Tools

The tools section of the problem formulation should indicate what software will be used for the risk assessment and may include why the software was chosen. The tools list should also include mathematical tools such as options for dose-response models.

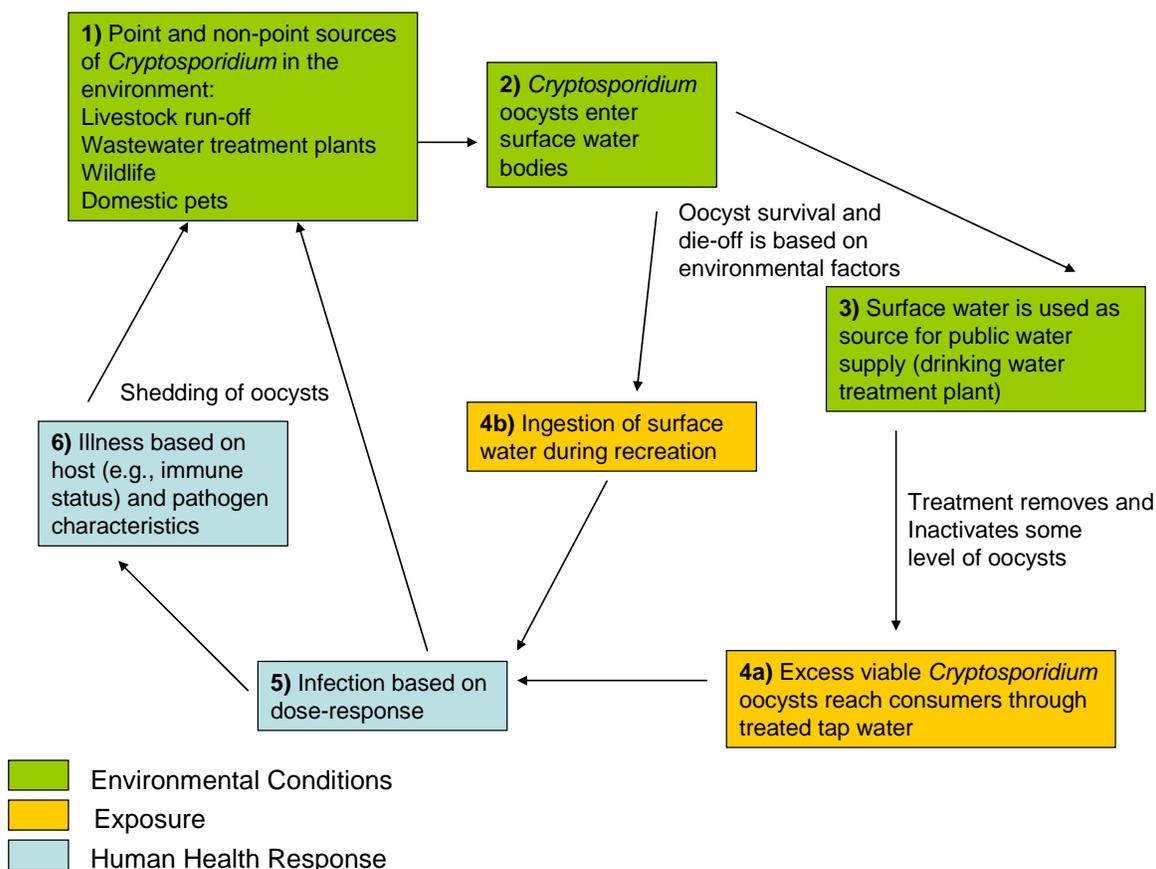


Figure 6. Example of an Overview (Top-Tier) Conceptual Model

“Tools” is also an appropriate section to describe the methods that will be used for dealing with uncertainty. Other types of methodological tools can also be presented.

Data Inventory

The data inventory should list publications that might be consulted during the risk assessment process and sources of data that are being considered for the risk assessment. The list does not need to be comprehensive in the beginning and can be presented in an appendix of the problem formulation if it is overly long. The data inventory may be a work in progress throughout the risk assessment. The data inventory can refer to a literature search strategy that can be presented in an appendix.

Summary of Assumptions

The summary of assumptions can be organized in different ways; however, listing assumptions that are related to essential risk assessment factors is a systematic way to start. How assumptions limit the scope of the risk assessment and contribute to uncertainty should be explained. The assumptions can be modified and updated as the risk assessment develops.

Sources of Variability and Uncertainty

The sources of variability and uncertainty should be introduced in this section, which should also describe the degree to which variability and uncertainty is or is not captured in the assessment. The iterative nature of problem formulation allows this list to be modified as the risk assessment scope is defined.

Factors and Data not Included and Explanation of Why

There may be information that is not utilized or avenues not pursued in the risk assessment. The explanation for not including that information should be presented, particularly if other related or similar types of risk assessments have included the information.

Identified Gaps in the Knowledge Base

Although gaps and data limitations may be noted throughout problem formulation they should also be summarized. Gaps can include a lack of adequate analytical or statistical methods and/or appropriate data and data quality. The summary of knowledge gaps can be useful for prioritizing future resource allocation (e.g., research and development needs) within the context of the results of the risk assessment. Knowledge gaps and data limitations can also affect the number and type of assumptions used in the risk assessment.

Environmental Sampling Strategies and Analysis Methods

Any issues associated with environmental sampling and analysis should be outlined during problem formulation so they can be fully considered during risk characterization. For microbial enumeration, issues may include percent recovery from different sample matrices and the ability

of a method to determine viability. The accuracy, precision, and biases should be included in the description of the methods and protocols.

Intervention Action Options

Depending on the scope of the risk assessment, it may be appropriate to identify which components in the risk assessment could influence or be influenced by management actions. It may be desirable to incorporate scenarios in the risk assessment that include intervention actions.

2.2.9 Planning and Scoping – Operational Plan

The operational plan should include strategies for dealing with data needs, peer review plans, and any other relevant logistical needs. Information such as lists of relevant experts (for consultation or data contribution) and literature search strategies can be included. This plan may contain a risk assessment process diagram that is a graphical representation of the operational plan that helps explain the logistics of conducting the risk assessment. The plan can also outline proposed phases for the risk assessment as well. Other essential management activities that are part of planning and scoping include timelines, planned deliverables (e.g., status briefing memos, draft for peer review, final draft), team assignments, and possibly budget details. Planning and scoping activities beyond the core scientific issues of problem formulation may be referred to in the risk assessment if those details help increase understanding and transparency.

2.3 Factors to Consider During Problem Formulation

The problem formulation process should provide a working outline of the risk assessment. Furthermore, information that is required during the analysis phase⁹ of risk assessment (Figure 7) is preliminarily gathered and reviewed during problem formulation. The conceptual model for the analysis phase shown in Figure 7 follows the general outline for environmental risk assessments (EPA, 1998) and the EPA-ILSI Framework (ILSI, 2000). The black diamonds in Figure 7 indicate major areas of interaction and overlap. The elements also have the potential to influence one another. Where appropriate, these influences should be noted during problem formulation and included in the analysis phase.

Several small modifications have been made to the Analysis conceptual model and incorporated into this MRA Protocol based on suggestions from the EPA problem formulation workshop (EPA, 2003c). In the EPA/ILSI Framework, “pathogen characterization” (which as explained below has been changed to “infectious disease

⁹ “Analysis plan” is analogous to an operational plan, whereas the “analysis phase” encompasses the exposure characterization and the human health characterization (also known as dose-response assessment). The analysis plan includes logistical details for the entire risk assessment, not just the analysis phase. To avoid confusion, the term “analysis plan” is not used in this Protocol; however, the elements of analysis plans from other EPA documents are included in this Chapter.

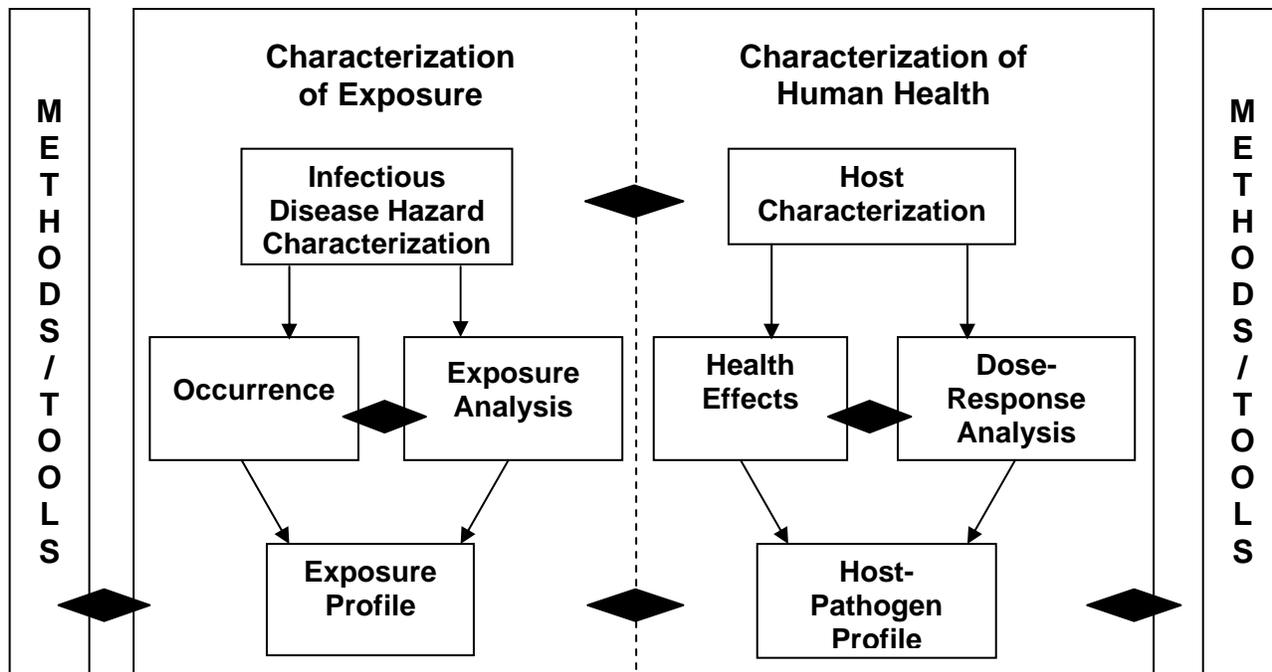


Figure 7. Analysis Phase Microbial Risk Assessment for Pathogens
 (Source: Adapted from ILSI, 2000)

hazard characterization”) and “host characterization” are considered part of the analysis phase of risk assessment (ILSI, 2000). In this MRA Protocol, the infectious disease hazard characterization and host characterization are initially considered as part of problem formulation because the resulting data and information are important for building the conceptual model(s) and making the decision if adequate data are available for the desired scope of a given risk assessment. It is appropriate to consider these steps as overlapping with the analysis phase because the data gathered during problem formulation are then used during the analysis phase.

2.3.1 Infectious Disease Hazard Characterization

The EPA-ILSI framework (ILSI, 2000) identifies pathogen characteristics that should be considered during problem formulation. These pathogen characteristics will influence both the exposure and health effects components of the risk assessment. Those considerations were expanded slightly during the EPA problem formulation workshop (EPA, 2003c) whereby the term “infectious disease hazard characterization” was recommended.¹⁰ An infectious disease hazard is broadly defined as any pathogen, indicator or surrogate that can be related to infectious disease occurrence. One example of an infectious disease hazard is indicator bacteria whose levels in recreational water can be related to illness in swimmers. An infectious disease hazard does not have to be the cause of illness (or whatever health endpoint is of concern), but should have a logical connection to the risk that is scientifically plausible. Infectious disease hazard can

¹⁰ Infectious disease hazard characterization occurs during problem formulation and is further elaborated during Characterization of Exposure. The term should not be confused with “hazard characterization” as defined by NRC and Codex approaches, both of which refer to the dose-response component of risk assessment.

also include multiple pathogens simultaneously, such as reported by Westrell et al. (2003), where risks due to failures in drinking water treatment systems were modeled for *Cryptosporidium*, rotavirus, and *Campylobacter jejuni*.

Factors related to infectious disease hazards that should be considered during problem formulation are listed and briefly discussed below.

Pathogen characterization elements include (adapted from ILSI, 2000):

- survival, multiplication, and accumulation;
- resistance to control or treatment processes; and
- ecology (including zoonotic potential, vectors, and epidemiological triangle).

Pathogen elements that overlap between exposure and human health effects include:

- virulence and pathogenicity of microorganism;
- pathologic characteristics/disease caused;
- host specificity (including zoonotic potential and vectors);
- infection mechanisms/route of infection/portals of entry;
- potential for secondary transmission; and
- taxonomy/strain variation.

Environmental Survival, Multiplication, and Accumulation

A microorganism (pathogen and or indicator) may be able to survive in water but be unable to infect a host. Many molecular-based microbial assays and some fluorescent antibody assays do not distinguish between live/dead or infectious/noninfectious organisms (e.g., deoxyribonucleic acid [DNA] amplification methods, polymerase chain reaction [PCR]). For assays that require growth of the microorganisms under laboratory conditions there is a concern that viable but non-culturable (VBNC) microorganisms will not be detected. Risk assessors should be aware of, and report the caveats of, the assays used to quantify microorganisms in the studies they use as data sources for an MRA.

Multiplication refers to the ability of some microorganisms to reproduce or grow in the environment. The combination of survival, viability, infectivity, virulence, and multiplication may be addressed through fate and transport modeling. Accumulation can occur in a variety of ways. Some examples include accumulation in biofilms (in pipes or tanks), accumulation in sediments, adsorption to particulate matter in water, and bioaccumulation in filter feeding aquatic organisms (e.g., shellfish). These factors contribute to heterogeneity of microbes. Places in the risk scenario where accumulation could occur should be noted.

Survival, multiplication, and accumulation are dependent on environmental conditions such as temperature, nutrient availability, and other water quality parameters. Treatment processes can also influence survival and may alter virulence and pathogenicity. Table 2 presents several representative tools for modeling pathogen survival, multiplication, and accumulation. Environmental niches that can harbor pathogens should be considered, such as biofilms and

Table 2. Representative Tools for Modeling Pathogen Survival, Multiplication, and Amplification

| Tools | Reference |
|---|---|
| USDA/ARS Pathogen Modeling Program (PMP) estimates the effects of multiple variables on the growth or survival of foodborne pathogens | Version 7.0 http://www.arserrc.gov/mfs/Download.htm |
| ComBase, also developed by USDA/ARS, is an on-line database of predictive microbiology information collected from researchers, institutions, and published literature. ComBase may be searched based on temperature, pH, water activity, condition, source (publication), organism, and environment. Files are provided giving organism, maximum rate, doubling time or D-value, source, conditions, environment, temperature, pH, water activity, a table and chart for log concentration versus time, and other available details. (Maximum rate is the maximum slope of the “log [cell concentration] versus time” curve, in a given environment.) | http://wyndmoor.arserrc.gov/combase/default.aspx |
| Continuous simulation | Recommended by TMDL Protocol (EPA, 2001) |
| Monte Carlo simulation | Recommended by TMDL Protocol (EPA, 2001) |
| Log-normal probability modeling | Recommended by TMDL Protocol (EPA, 2001) |
| <i>Survival and Transport of Viruses in the Subsurface: An Environmental Handbook.</i> The purpose of this issue paper is to discuss some of the conditions under which viral contaminants may survive and be transported in the subsurface, identify sources as well as indicators of viral contamination, outline the effects of hydrogeologic settings on viral movement, and introduce the reader to the current state of virus transport modeling along with an example of modeling applications. | EPA, 2003d |

amoebae (e.g., *Legionella* can live inside amoebae; Brown and Barker, 1999). The extent to which survival, multiplication, and accumulation will impact the risk assessment should be considered and documented during problem formulation.

Resistance to Control or Treatment Processes

Microorganisms have varying degrees of resistance to treatment and control processes. The extent to which these control or treatment processes will impact the risk assessment should be considered and documented during problem formulation. For example, data on how pathogens (or indicators) respond to both wastewater treatment and public water supply (PWS) treatment should be noted, as appropriate. If the risk assessment is for a performance target then the treatment and control processes may be of central importance. For example, *Cryptosporidium* oocysts are very resistant to (conventional) disinfection with chlorine, so treatment with chlorination in the absence of filtration may be inadequate to protect public health if oocysts are present in source waters.

Ecology

The epidemiological triangle (epi triad) is the recommended model for conceptualizing agent-host-environment interactions and is a useful way to consider ecology. The epi triad can be used to predict epidemiological outcomes and provides a tool to discuss parameters that influence public health outcomes. The epi triad can capture how pathogen, host, and environment all affect each other (Figure 8).

Physical properties of microorganisms that relate to their transport/mobility (e.g., hydrophobicity/hydrophilicity) and data on pathogen survival and bacterial colonization under varying ecological conditions (e.g., stressors such as pH, nutrient availability, and temperature) should be considered and discussed within the context of the scope of the risk assessment. Often, the ecology of pathogens can be elucidated by examining their transport, fate, and survival in the environment—particularly how they react in variable media. If sufficient data or information is available to prepare an ecology summary for the pathogen (or indicator), it should focus on the appropriate exposure source¹¹ (water, food, other) and may include environments that the microorganisms encounter before they enter the media of concern. For example, *Mycobacterium avium* complex thrive in hot water and have been known to colonize hot water systems in hospitals and buildings.

Ecological niches may also be provided by other microorganisms. For example, biofilms create ecological niches that are important to consider because microbes often different properties in communities compared to the same species living in suspension. For example, *V. cholerae* from human stool has enhanced infectivity relative to infectivity of dispersed planktonic cells in a rabbit model (Faruque et al., 2006). This is because *V. cholerae* from human stool is in conditionally viable forms (biofilms and multicellular clumps) that resist cultivation by conventional techniques but are more infective in a rabbit model than an equivalent number of planktonic cells.

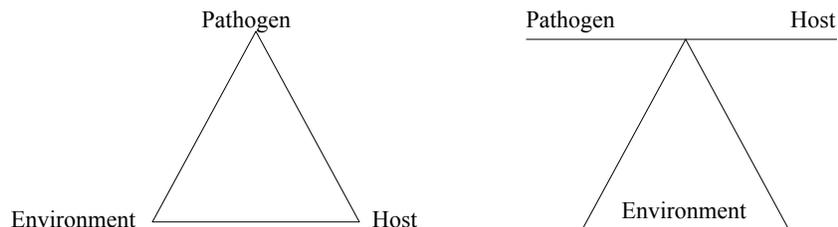


Figure 8. Two Versions of the Epi Triad
(Source: CDC, 1992)

¹¹ Exposure sources can be the media through which the contaminant is delivered, such as water or shellfish, or exposure sources can indicate the origin of the contaminant, such as point source, non-point sources, or naturally occurring. Exposure route indicates the sites of body contact that are relevant for access to sensitive tissues and organs, such as ingestion, inhalation, and dermal exposures.

Protozoa can also harbor bacteria within their cell membranes, therefore protecting the bacteria from many environmental stresses. *V. cholerae* occur commensally in zooplankton. A single copepod can carry up to 10^4 cells of *V. cholerae* and human volunteer studies show that $\sim 10^4$ to 10^6 *V. cholerae* can cause clinical cholera (Colwell et al., 2003). Legionella are known to reside within at least 20 species of amoebae, two species of ciliated protozoa, and one species of slime mold (Lau and Ashbolt, 2009). Species from the genera *Vibrio*, *Mycobacterium*, *Helicobacter*, *Afipia*, *Bosea*, *Pseudomonas*, and mimiviruses are also associated with protozoa in the environment (Lau and Ashbolt, 2009).

Virulence, Pathogenicity, Pathological Characteristics, and Host Specificity

Virulence and pathogenicity¹² refer to how easily and effectively a pathogen may cause disease in a host. Although both can be expressed numerically, the general definitions can be broader. Pathological characteristics are a description of the disease symptoms that result from exposure and infection by the pathogen (including strain variations). The known range of disease symptoms should be briefly reviewed and the specific health endpoint that the risk assessment addresses should be presented within the context of the broader range of health endpoints.

Host specificity is a pathogen characteristic that is related to host susceptibility. A species is not considered a host if it cannot be infected by the pathogen. A species can still be considered a host even if no illness results from infection. Within a host species there is variability in susceptibility. For example, mild illness could occur in immunocompetent persons resulting from exposure to a pathogen whereas severe illness may occur in immunocompromised persons. However, host specificity most often refers to the range of species that are infected by the pathogen. Information that can facilitate the comparison of human response to a pathogen versus laboratory animal models' response should be examined and is particularly important if data from animal models will be used to characterize dose-response or symptomatology during the risk assessment. Wild and domestic animals may also be prone to infection and disease (zoonotic potential) and thus may be a source of pathogens for human exposure either directly or through transport in the environment. For some pathogens there are non-human carriers, also known as vectors, which are important in the pathogen life cycle or serve as an environmental reservoir.¹³ The potential role of susceptible animals, vectors, and environmental reservoirs in the risk scenario should be addressed, which may include an explanation of how animals are contaminating the water sources of concern. These factors are also evaluated in greater detail in the health effects section of the risk assessment.

¹² Virulence is “the degree of intensity of the disease produced by a microorganism as indicated by its ability to invade the tissues of a host and the ensuing severity of illness.” Pathogenicity is “the property of an organism that determines the extent to which overt disease is produced in an infected population, or the power of an organism to produce disease. Also used to describe comparable properties of toxic chemicals. Pathogenicity of infectious agents is measured by the ratio of the number of persons developing clinical illness to the number exposed to infection” (EPA, 2007a).

¹³ The term “vector” can mean “anything which transmits parasites” (for this report a vector can transmit bacteria, virus, or parasite) (www.swintons.net/jonathan/Academic/glossary.html) or can refer to intermediate hosts that are required for life cycle completion. Environmental reservoirs include free-living amoeba that can harbor bacteria intracellularly allowing the bacteria to survive in harsher environments than they could normally survive (NRC, 2004).

Infection Mechanisms, Route of Infection, and Portals of Entry

Infection mechanisms, route of infection, and portals of entry emphasize the manner in which pathogens interact with hosts. The exposure routes¹⁴ that will be included in the risk assessment are defined during problem formulation. Part of that definition should include identification of known routes that will not be part of the scope of the risk assessment. For example, in many waterborne pathogen risk assessments the ingestion route of exposure is investigated and other routes of exposure (e.g., inhalation) are not included. In cases where a pathogen is not known to be infectious through certain routes, such as the dermal route, the justification for not including the dermal exposure route should be included in the risk assessment documentation, particularly for risk assessments conducted to meet specific statutory requirements where reasons for excluding a route must be justified.

For an infection to occur, the target organ of the host must come in contact with a sufficient number of microorganisms, the microorganism must possess specific virulence factors, these virulence factors must be expressed, and the defenses of the host and/or target organ systems (e.g., digestive system, lung) must be overcome. With some microorganisms (e.g., *Giardia*, *Cryptosporidium*) the interaction with the particular organ is so specific that infections are almost always confined to that one organ site; with others (e.g., *Salmonella*, enteroviruses) the pathogen has the potential to become systemic and may be able to initially infect more than one target organ. When attempting to establish a health risk due to exposure to pathogens through contact with food and drinking water, one must consider that the human gastrointestinal (GI) tract is a complex organ system with a variety of specific host defense mechanisms. It is only when the pathogen has particular virulence factors for sites in the GI tract, and the specific host defense mechanisms in the GI tract are breached, that infection occurs. Infection without symptoms and the duration of infection are important attributes of the infectious process because they contribute to the potential for secondary transmission via the shedding of pathogens into the environment.

Secondary Transmission

The potential for secondary transmission will also contribute to human exposure. Secondary transmission refers to infection spreading from one infected person to another person. Secondary cases (often represented by a secondary attack rate) generally refer to cases or an attack rate that occurs among contacts, within the incubation period of the pathogen, and following exposure to a primary case. In some cases, direct person-to-person transmission cannot be separated from contamination of the immediate environment and subsequent transmission to another person (e.g., toddlers sharing toys versus direct physical contact during play). In most cases, it is appropriate that the definition of secondary transmission include infections that result from propagation of the specific exposure of interest, but not encompass distant transmissions (separated by time and/or space) that may be more appropriately considered to result as a function of person-to-environment-to-person transmission. Temporal and spatial limitations should be specifically noted in the definition of secondary transmission for a given pathogen.

¹⁴ Route of exposure refers to how the pathogen comes in contact with the vulnerable host receptor cells that support infection (e.g., inhalation, dermal contact, oral), whereas source of exposure refers to the physical matrix that carries the pathogen (e.g., air, water, food, soil).

Full discussion of the range of scenarios that qualify as secondary transmission should be included where appropriate. The above definition of secondary transmission is limited to avoid overlap with pathogen occurrence in the environment (person-environment-person), although people are, of course, part of the environment. However, the potential for re-introduction of the pathogen into the exposure media could also be within the definition of secondary transmission. Dynamic MRA models can characterize secondary cases that occur among contacts following exposure to a primary case, whereas static MRA models usually consider secondary transmission to be negligible or include it as a non-fluctuating multiplicative factor (e.g., secondary cases equal primary cases multiplied by 0.1; assuming a 10% secondary transmission rate). The problem formulation documentation should indicate if/how secondary transmission is included in the assessment. If it is not included, justification for this decision should be provided.

Taxonomy and Strain Variation

Taxonomy and strain variation have a potentially large impact on risk assessment. The difference in dose-response range between isolates (and strains) can be orders of magnitude (see Text Box 4). Some strains may not be infective for humans. In addition, the ratio of different strains in the environment can fluctuate. These factors make characterization of pathogen occurrence difficult (Messner et al., 2001; Teunis et al., 2002). The extent to which strain variation is accounted for in the risk assessment should be documented during problem formulation.

2.3.2 Initial Host Characterization

Host characterization involves an evaluation of the intrinsic and acquired traits that modify the risk of infection or illness in a potentially exposed human population. It is also possible that host factors may be important in determining the severity or outcome of an infection. For example, high-risk groups may develop severe symptomatic illness, whereas low-risk groups may develop asymptomatic infections or mild illness.

The following populations are typically considered more susceptible¹⁵ than the general population: pregnant women; neonates and children; elderly (over 65 years of age); individuals residing in nursing homes or related care facilities; and cancer, organ transplant, and AIDS patients (Haas et al., 1999). The Report to Congress, *EPA Studies on Sensitive Subpopulations and Drinking Water Contaminants* (EPA, 2000d) summarizes EPA's approach to identifying and characterizing susceptible subpopulations that may be at greater risk from exposure to drinking water contaminants than the general population.

¹⁵ Sensitive subgroups are "identifiable subsets of the general population that, due to differential exposure or susceptibility, are at greater risk than the general population to the toxic effects of a specific air pollutant (e.g., depending on the pollutant and the exposure circumstances, these may be groups such as subsistence fishers, infants, asthmatics, or the elderly)." (EPA, 2007a)

Susceptible subgroups "may refer to life stages, for example, children or the elderly, or to other segments of the population, for example, asthmatics or the immune-compromised, but are likely to be somewhat chemical-specific and may not be consistently defined in all cases." (EPA, 2007a)

Note that in the above definitions "susceptible" refers to host characteristics and "sensitive" refers to host characteristics and exposure patterns.

Text Box 4. Virulence of Three *Cryptosporidium parvum* Isolates in Healthy Adult Humans

(Source: Okhuysen et al., 1999)

| Isolate | Iowa | UCP | TAMU |
|-------------------------------------|-------------|--------------|-------------|
| Infectious dose (ID ₅₀) | 87 oocysts | 1042 oocysts | 9 oocysts |
| Attack rate | 86% | 52% | 59% |
| Duration of symptoms | 64.2 hours | 81.6 hours | 94.5 hours |

Host characteristics have the potential to influence both the exposure and the health effects components of the risk assessment. These factors are often used to define potential subpopulations of interest for a risk assessment because they can influence the assessment with respect to susceptibility to infection and severity of illness. Factors related to host characterization that should be considered during problem formulation include the following and are briefly discussed below:

- immune status;
- age;
- concurrent illness/medical treatment (physical and mental stressors may increase susceptibility);
- genetic background;
- pregnancy;
- nutritional status;
- previous exposure (may confer protective immunity); and
- social/behavioral traits.

The extent to which these factors are considered in the risk assessment should be described in the problem formulation documentation.

Age,¹⁶ concurrent illness, medical treatment, genetic background, pregnancy, nutritional status, and previous exposure all have the potential to affect immune status.

- The young and the elderly generally have less resistance to infections. Children, especially malnourished children, may be more likely to exhibit severe effects of AGI after exposure to some pathogens (e.g., pathogenic *E. coli*, some enteric viruses). However, some pathogens (e.g., Hepatitis A and poliovirus) may cause less clinical illness in children than in adults (Gerba et al., 1996a). Age can also contribute to different exposure patterns due to behavior. For example, children may have higher levels of incidental ingestion of water during swimming than adults (Dufour et al., 2006). For drinking water consumption increases with age, so the elderly consume more drinking water than adults or children (Roseberry, and Burmaster, 1992)
- Populations that are considered immunocompromised or immunosuppressed due to

¹⁶ Although children are referred to in conjunction with subpopulations in this document, EPA acknowledges that childhood represents a life-stage rather than a subpopulation, the distinction being that a subpopulation refers to a portion of the population, whereas a life-stage is inclusive of the entire population (<http://www.epa.gov/teach/index.html>).

recent or concurrent illness or medical treatment may be defined as subpopulations which the risk assessment will address (Effler et al., 2001). However, all definitions of subpopulations included in the risk assessment should include the criteria used to classify individuals as immunocompromised and may need to be limited to specific identifiable types of immune defects.

- Genetic background can also affect immune status, but may play a larger role in mechanism of infection and disease progress.
- Pregnancy may cause women to be more susceptible to a pathogen. For example, Hepatitis E, which is a self-limiting disease for most people, can cause up to 20% mortality in women in the third trimester of pregnancy (Jameel, 1999).
- Malnourished individuals tend to have weaker immune defenses than well nourished individuals.
- Previous exposure may confer limited and/or short term protective immunity for some pathogens (Frost et al., 2005). The converse of this may also be true, that is, when individuals or populations that have not previously been exposed to particular pathogens, infection and illness rates can be higher than would otherwise be anticipated. “Traveler’s diarrhea” is an observed phenomenon that exemplifies this type of situation.
- Extreme physical or emotional stress can lower immune competency.
- The host GI environment can vary in ways that affect pathogens and innate immunity also plays a role in infection dynamics (Text Box 5).

Social and behavioral traits primarily affect exposure patterns. For example, a relatively small proportion of the population is responsible for consuming the majority of raw and partially cooked shellfish (FDA, 2001). As mentioned above, age may also be related to behaviors that affect pathogen exposure patterns.

In terms of addressing the above factors in a risk assessment, data for the above elements can be arranged into groups by stratification or multivariate analysis. Alternatively, host characteristics can be considered by conducting a separate risk assessment for each characteristic that is believed to have some importance. For example, in addition to a risk assessment for the overall population, a separate risk assessment may be performed for each subpopulation of interest (e.g., young children, the elderly, pregnant women, immunocompromised persons) provided that sufficient data are available for valid statistical interpretation. EPA’s Risk Assessment Forum has developed *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants* (EPA, 2005b), which recommends subgroups that address anatomy/physiological development in the following age groupings: birth to <1 month, 1 to <3 months, 3 to <6 months, 6 to <12 months, 1 to <3 years, 3 to <8 (female) or <9 (male) years, and 8 or 9 years to <16 (female) or <18 (male) years.

In recognition that children have a special vulnerability to many toxic substances, the EPA Administrator’s *Policy on Evaluating Health Risks to Children* (October 1995) directs the Agency to explicitly and consistently take into account environmental health risks to infants and children in all risk assessments, risk characterizations, and public health standards set for the United States. In April 1997, President Clinton signed Executive Order 13045 *Protection of Children from Environmental Health Risks and Safety Risks*, which assigned a high priority to addressing risks to children (EO, 1997). In May 1997, EPA established the Office of Children’s

Text Box 5. Host Immune Responses

Much more is known about human immune responses than can or should be incorporated into most risk assessments. However, during literatures searches for data and background, risk assessors should be alert for new scientific developments that may be appropriate to incorporate into the risk assessment model, while keeping in mind that scoping the risk assessment should exclude information that is not necessary for answering the questions posed during problem formulation.

For example, much is known about GI-associated lymphoid tissues, which provide host defenses for mucosal tissues (Acheson and Luccioli, 2004; Forchielli and Walker, 2005; Wershil and Furuta, 2008). Host factors such as membrane-bound “toll-like” receptors and cytoplasmic nucleotide-binding proteins detect microbial pathogens and trigger innate immunity responses that are essential for controlling GI pathogens such as *Salmonella*, *Helicobacter pylori*, and *Listeria monocytogenes* (Eckmann, 2006). The cytokine gamma interferon is important in immune responses to *Cryptosporidium* in mice (Gomez Morales and Pozio, 2002; Rogers et al., 2006). Also, flora present in adult mouse intestinal mucosa can transfer resistance to *C. parvum* when fed to susceptible infant mice (Harp, 2003). Mucosal immunity is an important factor in *Cryptosporidium* infection in calves (Wyatt, 2000). Knowledge of immune system modeling in response to pathogens is also being advanced (Marino et al., 2007). Thus, further advances in knowledge about host-pathogen interactions in complex GI ecosystems may be relevant to some risk assessment models in the future.

Health Protection to ensure the implementation of the President’s Executive Order. EPA has increased efforts to ensure its guidance and regulations take into account risks to children. In 2002, EPA published an interim report on child-specific exposure factors (EPA, 2002d).

Environmental Justice (EJ)

Executive Order (EO) 1289, *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations* (February 1994), ordered Federal agencies, including EPA, to “...make achieving environmental justice part of its mission by identifying and addressing, as appropriate, disproportionately high and adverse human health or environmental effects of its programs, policies, and activities on minority populations and low-income populations...” (EO, 1994). EPA responded to E.O. 12898 with *The EPA’s Environmental Justice Strategy* (EPA, 1995c). In 2001, in a memo from the EPA Administrator EJ was defined as follows: “The Agency defines environmental justice to mean the fair treatment of people of all races, cultures, and incomes with respect to the development, implementation, and enforcement of environmental laws and policies, and their meaningful involvement in the decisionmaking processes of the government.” (emphasis in original).¹⁷ EPA further defined meaningful involvement in EPA’s *Public Involvement Policy* (EPA, 2003g) as follows:

¹⁷ <http://earth1.epa.gov/oswer/ej/html-doc/ejmemo.htm>

“Meaningful involvement”...means that: (1) potentially affected community residents have an appropriate opportunity to participate in decisions about a proposed activity that will affect their environment and/or health; (2) the public's contribution can influence the regulatory agency's decision; (3) the concerns of all participants involved will be considered in the decision-making process; and (4) the decision makers seek out and facilitate the involvement of those potentially affected.

Risk assessment documentation should provide clear descriptions of subpopulations and other parameters that may help EPA evaluate whether there are potential environmental disparities that could cause an EJ concern.

3. Exposure

The risk assessment analysis phase consists of the technical evaluation of data concerning the potential exposure and associated health effects (also referred to as dose-response assessment for pathogens or exposure-response assessment for indicators or other hazards). Although problem formulation may partially address many of the issues to be evaluated in the analysis phase, the analysis phase is generally more detailed and quantitative. The exposure and human health components of the analysis phase can be mapped out during problem formulation. Chapter 3 discusses Exposure Assessment while Chapter 4 discusses Health Effects (dose-response assessment). Characterization of exposure and human health effects are iterative and inter-related processes because they must be compatible for use in the final risk characterization.

In human health risk assessment, *exposure* is defined as human contact with a biological, physical, or chemical agent, usually through ingestion, inhalation, or dermal contact. Risk assessment can be performed for specific target populations or an individual target organism (a human with a defined exposure pattern). Exposure assessment involves the determination or estimation (qualitative or quantitative) of the magnitude, frequency, duration, and route(s) of exposure (EPA, 1997a). A primary purpose of exposure estimation is to support dose estimation (EPA, 1992). *Dose* is the amount of a pathogen that enters or interacts with a host (ILSI, 2000).¹⁸

For nearly all microbial risk assessment contexts, dose refers to potential dose (i.e., the number of pathogens ingested in a specified period.) Note that risk assessments can also be performed without direct estimates of pathogen dose. Risks can be calculated as a function of pathogen concentration in the exposure medium (e.g., drinking water), without the intermediate step of dose calculation. An important reason for calculating pathogen doses is that doing so allows the data from one exposed population (e.g., the volunteers in a virulence study) to be applied to risk assessments for other exposed groups, such as the general population.

Characterization of exposure involves an evaluation of the interaction between the pathogen, the environment, and the human population (i.e., the classic epidemiological triad, Figure 8). The Infectious Disease Hazard Characterization, Occurrence, and Exposure Analysis sections are brought together (see Figure 7) to develop an Exposure Profile that quantitatively or qualitatively

¹⁸ EPA (EPA, 1997a, 2003e, 2004b, 2005a) has defined “*dose* as the amount of a substance available for interactions with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. The *potential dose* is the amount ingested, inhaled, or applied to the skin. The *applied dose* is the amount presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism). The *absorbed dose* is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of the skin, lung, and digestive tract) through uptake processes. *Internal dose* is a more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The amount of the chemical available for interaction by any particular organ or cell is termed the *delivered* or *biologically effective dose* for that organ or cell.” Note that these sub-definitions of dose are not used to describe pathogen interactions with hosts. The term “minimum infectious dose” was intended to indicate the lowest dose that would cause infection in an individual and assumed that there was a threshold dose. This term is generally considered to be obsolete because as little as one microorganism is believed to be capable of causing infection in a susceptible individual. However, it should be reiterated that infection does not imply symptomatic illness (EPA, 2007a).

summarizes the magnitude, frequency, and pattern of human exposure for the scenario(s).

Exposure is not limited to a pathogen-specific context. It can also be defined in terms of water quality indicators such as the presence of coliforms, pathogen surrogates, or types of water sources (e.g., ground water, impoundments, rivers) coupled with estimated efficiencies of treatment technologies (e.g., filtered and disinfected water versus disinfected water only). In all cases where indicators are used in risk assessment, it is important to document fully the basis for their use (i.e., the extent to which they are correlated with the pathogen or health effect of concern), and to specify clearly the conditions under which the correlation is expected to be valid.

3.1 Occurrence

Occurrence refers to the conditions that lead to the presence of a hazard or to the distribution pattern of an agent (e.g., pathogen, indicator) in the environment and the media of concern. The EPA-ILSI Framework (ILSI, 2000) identifies the following elements to consider when characterizing pathogen occurrence during exposure assessment:

- temporal distribution/frequency;
- concentration in environmental media;
- spatial distribution (clumping, aggregation, particles, clustering);
- niche (ecology, non-human reservoirs);
- survival, persistence, amplification;
- seasonality;
- meteorological and climatic events;
- presence and effectiveness of treatment or control processes; and
- indicators and surrogates and relationships.

Many of the factors listed above are interrelated and as such cannot be discussed independently. There are three basic questions that should be answered to describe pathogen occurrence in a water body—when (including duration), where, and how much (level)? When information on a particular pathogen species of interest is lacking, it may be necessary to use occurrence data for surrogate or indicator species. The limitations and uncertainty associated with those data and their use should be evaluated and discussed.

When Do Pathogens Occur in the Water Body?

Temporal distribution/frequency describes when pathogens occur. Fluctuations in pathogens can occur on almost any time scale (Boehm, 2007; Boehm et al., 2002). For many pathogens, data are available for characterizing hourly, daily, weekly, monthly, seasonally, or yearly fluctuations. Meteorological and climatic events such as storms may precede changes in pathogen occurrence. Seasonality is a factor that affects the temporal frequency of most waterborne pathogens, such as *Cryptosporidium*. Seasonal events, such as calving or bearing young seem to be associated with some zoonotic pathogens. Occurrence data that are linked to temporal events such as storms or seasons may be useful for predicting how pathogen levels may respond to future events. If wastewater treatment or control processes are not consistent, such as may occur during storm

events (combined sewer overflows and sanitary sewer overflows), there may be associated temporal fluctuations in pathogen levels. Urban and agricultural runoff can also influence pathogen occurrence in surface waters.

Where Do Pathogens Occur in the Water Body?

Spatial distribution of pathogenic microorganisms can differ depending on the microorganism and on the properties of the water matrix. If pathogen occurrence fluctuates over time, then the degree of clumping, aggregation, and clustering may also change as water parameters change. Unlike chemicals, pathogens are particulates and may stick to each other or sediment and other particles (Gerba et al., 1991). The size and nature of particles will influence suspension and settling in different hydraulic conditions. Therefore, particles that carry pathogens may be distributed within a water body in an uneven (heterogeneous) manner. For waterborne pathogens, niche is relevant for “free living” species. Pathogens may thrive in open water, sediments, or other ecologically defined spaces. Non-human reservoirs may also be an important part of the pathogen’s ecology. If there is appreciable survival, persistence, or amplification in non-human species, then those sources of contamination of water may need to be considered during the occurrence assessment. This includes animals (wildlife and domestic) as well as other microorganisms. Survival, persistence, and amplification can differ in different microclimates within a water body, and should be considered factors that influence where pathogens occur. Pathogens in sediments may be resuspended in the water column due to changes in flows associated with precipitation, runoff, tides, and currents.

What is the Level of Pathogens in the Water Body?

Pathogen levels will vary across time and space. Characterizing pathogen occurrence relies on measuring the concentration in the environment and correlating concentration with spatial and temporal patterns in the environment, such as niches, seasons, weather events, and human-related activities. There are several difficulties that are commonly encountered when measuring pathogen levels in water samples. Because microorganisms tend to clump and aggregate (heterogeneous distribution), replicate samples can yield measurements that differ substantially (even by orders of magnitude). Assays used to quantify pathogens yield variable recovery rates and may or may not include information about the viability of the pathogens or their infectivity to humans. These factors could be important when monitoring for treatment efficacy. For example, the excystation assays used to indicate *Cryptosporidium* viability resulted in the conclusion that ultraviolet (UV) light irradiation was not an effective treatment option for *Cryptosporidium*. This is because low UV doses do not significantly affect oocyst excystation. However, *in vivo* assays indicate that for *Cryptosporidium* treated with low UV doses, infection does not progress (Bukhari et al., 1999; Clancy et al., 2000; Craik et al., 2001). It is important to document measurement techniques and their capabilities and limitations carefully so that scientifically defensible decisions can be made about integrating or not integrating results from different studies.

The ability for a pathogen to survive and also remain infectious in a water body is dependent on both pathogen characteristics and environmental factors. Pathogen-specific characteristics include but are not limited to, genetic strain variations, the growth conditions the pathogen

experienced before entering the water body, duration in the environment, protective states, and VBNC states. Environmental factors include but are not limited to, temperature, pH, turbidity, nutrient levels, osmotic conditions, UV exposure, predation, and interactions with other living organisms. For example, and as noted previously, amoebae may be reservoirs that may contribute to the survival, persistence, and/or amplification of environmental pathogens such as *Legionella* species, some *Salmonella*, and some *Mycobacteria* species. Fate and transport modeling can provide plausible scenarios and estimates of how microbial concentrations can change over time as they move through the aquatic environment.

Pathogen and indicator occurrence patterns will also be affected (but not necessarily in a similar manner) by the presence of control strategies and treatment processes (either wastewater or drinking water treatment depending on the context). Mitigation strategies may involve improving existing control processes or adding new control measures, which can be modeled in the risk assessment. Discussion of the sources of microbes may be helpful in characterizing occurrence patterns. Some commonly considered sources include, wastewater treatment plant effluent, some industrial effluents, leaking septic tanks, urban run-off, agricultural run-off, animals (e.g., livestock, domestic, wildlife), and environmental niches (e.g., sediments, aquatic plant life). Concentrations of pathogens vary in untreated sewage based on the level of shedding in the contributing human population and concentrations in treated sewage vary based on levels before treatment and efficacy and type of treatment processes. Differences in contributing populations can result in orders of magnitude differences in microbial levels in sewage. For example, sewage from developing countries can have 100-times higher levels of enterovirus than sewage in the United States. (Gerba et al., 2008).

The outcome of the “occurrence” section of the process is an evaluation of all relevant factors pertaining to the occurrence and distribution of the pathogen or indicator. Several tools and databases for evaluation of occurrence, which may be useful for microbial risk assessment exposure scenarios, are summarized in [Appendix F](#).

Because “infectious disease hazard” is broadly defined and can include behaviors or scenarios that do not directly relate to pathogens, the factors that influence the occurrence of infectious disease hazards may be unique to the hazard. For example, in a risk assessment that considers combined sewer overflows to be the infectious disease hazard, the occurrence of the hazard would be linked to factors such as rainfall and geographical information on locations of outfalls from sewer maps.

3.2 Exposure Analysis

An exposure scenario summary can be a short narrative description of how an individual is exposed to a hazard. A more formal exposure scenario provides additional detail about the range of exposures that are considered in the risk assessment. The EPA *Exposure Factors Handbook* (EPA, 1997a) is the Agency-wide resource for building exposure scenarios for chemical hazards. It can also be consulted for data that may apply to infectious disease hazard exposures. Elements that should be evaluated for inclusion in exposure analysis and are used to define the exposure scenario scope (and which can apply to pathogens or indicators) are presented below (adapted from ILSI, 2000).

Exposure-related:

- identification of media;
- units of exposure (period of relevancy to characterize dose);
- routes of exposure (including secondary transmission); and
- spatial and temporal nature of exposure (whether single or multiple exposures).

Characteristics of exposed population:

- size of exposed population;
- demographics of exposed population; and
- behavior of exposed population.

3.2.1 Identification of Media

Identification of media in this MRA Protocol refers to the specific water sources being considered in the risk assessment. In the context of risk assessment for CWA 304(a) AWQC, the “designated use” determines the exposure scenarios that are considered. Finished drinking water is usually considered separately from source waters. Surface water may be considered separately from ground water, and marine recreational waters may be considered separately from fresh recreational waters. MRAs for biosolids-related exposures have been conducted in the same manner as described herein for water-related exposures (Eisenberg et al., 2004, 2008; Gale, 2003, 2005);

3.2.2 Units of Exposure

The unit of exposure is generally “dose per specified time span” (e.g., the number of organisms a person is exposed to per exposure event). For pathogens, exposure events are usually measured on a time scale of daily or shorter intervals. Historically, exposure time frames for pathogens have been based on the assumption that short-term (event-based) exposures are most relevant (e.g., per swimming event for recreational activities; per day for drinking water uses) rather than lifetime exposures. In contrast to chemical contaminants in water, the adverse health effects associated with human exposure to waterborne pathogens have been best documented for event-related (short-term, single exposure) rather than chronic exposure over long periods of time. These short-term exposure timeframes have been used because infection requires one or more pathogens to be ingested and that at least one of the ingested pathogens succeeds in establishing itself in or on cells somewhere within the GI tract of the host (Teunis and Havelaar, 2000). If no organisms have been ingested or none of those ingested succeed in passing all of the host barriers, infection does not occur. Note that short-term exposures do not necessarily imply that only short-term or minor adverse human health effects occur; for example, illnesses from some pathogens can be severe and/or long-term or produce sequelae (Rangel et al., 2005).

Most MRAs do not address the probability of exposures to microbes over a lifetime. Although there are chemicals for which a single exposure model is appropriate, such as teratogens that cause developmental defects or nitrate that can cause infantile methemoglobinemia, many cancer-causing chemicals exhibit increasing risk as duration of exposure lengthens (i.e., exposure

over multiple years). This is because some chemicals can accumulate in the human body, and even for chemicals that can be purged from the body, the damage they cause may not be readily repairable. Therefore, damage may accumulate with each subsequent exposure. Although accumulating damage is not necessarily an outcome of infection by pathogens, there are pathogens that generate toxins that behave similarly to chemicals in this respect. Therefore, it may be important to consider the mechanism by which pathogens cause illness symptoms when considering whether short-term, event-based exposures are the predominant relevant exposure pathway. Reinfection may also increase the potential for development of autoimmune disease. For example, the autoimmune disease, reactive arthritis, can be triggered by the following pathogens *Chlamydia*, *Salmonella*, *Shigella*, *Yersinia*, *Brucella*, *Leptospira*, *Mycobacteria*, *Neisseria*, *Staphylococcus*, and *Streptococcus* (Girschick et al., 2008).

Because for many pathogens, residence time in the human body, if infection does not take place, is unknown, it is difficult to determine specifically what exposure timeframe constitutes a “dose.” In addition, previous exposures to a pathogen typically do not result in increased risk of damage as is common for carcinogens, except where noted above for toxins. For some pathogens, previous exposure may even provide additional protection from that pathogen as a result of increased host immunity (Soller and Eisenberg, 2008).

The exposure timeframe basic unit should be discussed within the context of the exposure scenarios. It may be difficult to determine if recurring exposure events are completely independent or not. For example, microbial risk assessments for drinking water commonly assume that all water consumed over the course of a single day is considered to be one dose, and consumption on subsequent days are independent events (EPA, 2006a). When exposures are considered to be completely independent (e.g., consumption on different days) the cumulative risk can be calculated as the result of independent repeated risk events. At the other end of the scale, when exposures are considered to be completely dependent, the doses can simply be added and treated as a single risk event (e.g., add the volume of water consumed through each serving of water over the course of a day). However, little data are available to describe the mechanisms of pathogen infection processes to support the assumption that all consumption within a specified period constitutes a single exposure event. Instead, the 24-hour timeframe is used for convenience and because it is a biologically reasonable timeframe for human digestive processes. The interdependence of exposure events may be important for some pathogens and may vary depending on characteristics of the host, the pathogen, and event specific conditions such as delivery matrix. Although exposure events may have varying interdependence, the assumption of independent exposure events as a default assumption is commonly used in MRA. The uncertainties associated with defining the unit of exposure should be discussed in the risk assessment.

Some examples of units of exposure are presented in Text Box 6 below. Note that edible crop irrigation, aquaculture, and other water uses would likely have different units of exposure.

3.2.3 Routes of Exposure

The primary route of exposure¹⁹ considered in water-based microbiological risk assessment is

¹⁹ Route of exposure refers to how the pathogen comes in contact with the vulnerable host receptor cells that support

Text Box 6. Examples of Units of Exposure

- For recreational exposures, the unit of exposure may be per swimming day or hours spent in the water. Alternatively, risks from recreational exposures may be calculated using estimates of volume of ambient water incidentally (inadvertently) ingested over a given length of time (e.g., 50 mL/hour).
- For drinking water, the unit of exposure is typically a daily volume, for example, 2 L/person-day (which represents the 86th percentile for consumption from the 1994-1996 Continuing Survey of Food Intake by Individuals [CSFII] database) (EPA, 2006a).
- For shellfish, the unit of exposure can be a meal or serving (without including how much constitutes a meal), number of shellfish consumed per meal, or weight of shellfish consumed per meal.

usually ingestion. Inhalation exposures may be significant for some microorganisms (spore-forming bacteria, *Legionella*, MAC, and some viruses), but inhalation exposures are rarely considered in MRAs for waterborne pollutants. Similarly, dermal exposures (through intact skin or, more frequently, open cuts and scratches) may be important for some scenarios and may be considered (e.g., *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*). However, dermal exposures are rarely considered in quantitative microbial risk assessments for waterborne pathogens because dose response data is generally lacking for agents causing water related infections through dermal exposures. Unless an evaluation of the available data indicates that inhalation or dermal exposures of pathogens in water are important exposure routes, it may not be necessary to include them. For example, inhalation route would be appropriate for the enteric virus, Coxsackievirus, because respiratory pathway dose-response data are available but oral pathway data are not (Couch et al., 1965). Pathogens present in water could reach the lungs by inhalation of vapors coming from a water body, if a person inhales water directly, from showers, misters, or aerated taps.

Many water-based microbiological risk assessments focus on exposure through drinking water, and recreational activities such as swimming and other activities where ingestion of water is likely. Quantitative data have been developed to characterize the volume of water that individuals and/or populations ingest during these types of activities (Dufour et al., 2006; EPA, 1997a, 2002d, 2006). However, there are also other routes of exposure that also could be of interest for which less data are currently available, such as secondary and non-contact activities such as boating and fishing. Routes of exposure should be discussed in the risk assessment documentation, including which routes are considered and which routes are not part of the scope of the risk assessment.

3.2.4 Spatial and Temporal Nature of Exposure

The spatial nature of the exposure for waterborne pathogens is suggested by the CWA designated use. For example, exposure during recreation (swimming, surfing, etc.) occurs while people are on or in the water and is geographically confined to where the water body is located. Exposure through drinking water is limited to the area that the public water supply serves, unless water is

infection (e.g., inhalation, dermal contact, oral), whereas source of exposure refers to the physical matrix that carries the pathogen (e.g., air, water, food, soil).

transported (e.g., transported by truck to a neighboring community to fill a special need). People may also move between water districts throughout the day as they travel for work or other reasons. Exposure through consumption of raw or partially cooked shellfish can also occur at locations removed from the water body from which the shellfish originated. Complex spatial distributions of exposure and the exposed people can make characterizing exposure patterns difficult; however, those patterns should be analyzed for their affect on the exposure assessment.

The temporal nature of the exposure is included in the unit of exposure definition. Whether single or multiple exposures are considered should be discussed during the problem formulation stage. Risk assessment tools for considering multiple exposures to a given pathogen are currently not developed sufficiently to recommend any specific tools.²⁰ A significant gap in our knowledge is what temporal spans need to be considered for a “dose,” and how variable are those time spans when different pathogens are compared.

3.2.5 Characteristics of Exposed Population

The size of the exposed population refers to the number of people who come in contact with the media of concern. The demographics and behavior of the exposed population can conceptually include many possible subgroups. Defining the subpopulations that will be considered is a key component of problem formulation. For example, if different age groups are considered, it is necessary to define the age groups in a way that is consistent with available knowledge about exposures and susceptibility. If exposure-response relationships for “children” derived from epidemiological studies are used, it is important to use the same definition (age range) of children as was evaluated in the epidemiological study, unless a case can be made that the uncertainty introduced as a result of applying data from one group to another group is acceptable. Subgroup differentiation is not necessary unless there is evidence for relevant differences between the subgroups.²¹ There should be scientific rationale presented for dividing subgroups as well as data that directly pertain to that subgroup or can be adjusted to address that subgroup. For example, it is unlikely that differentiating between 24 and 25 years-olds would provide any additional useful information for risk managers.

Subpopulations can be defined by their susceptibility (e.g., intrinsic factors such as immune status or related factors) or by behaviors (extrinsic factors) that may cause them to be highly exposed (e.g., lifeguards, surfers, tri-athletes, other competitive swimmers versus casual bathers). In particular, plausible extreme behaviors should be noted and the discussion should clarify to what degree individuals exhibiting those behaviors are addressed by the exposure scenario. Behaviors can also influence the routes of exposure and the spatial and temporal nature of exposure.

Specialized exposure scenarios, such as occupational exposures, can also be developed. This type of exposure consideration would most likely require that the risk assessment include both scientific and regulatory considerations. Risk assessments limited to occupational exposures to water that have caused infectious disease outbreaks are not common. However, there may be

²⁰ Methods for considering exposure to multiple pathogens are also lacking.

²¹ Note that risk assessments being performed as part of a statutory requirement may already have mandated subgroups.

some occupations that have frequent water exposures in which a microbial risk assessment may be of interest (e.g., lifeguards, wastewater treatment plant workers).

3.3 Exposure Profile

The exposure profile is a distillation of the most important information and data that is developed during the exposure component of the analysis phase. Whereas each of the components of the exposure analysis (occurrence, identification of media, units of exposure, routes of exposure, spatial and temporal nature of exposure, and characterization of exposed population) describe as comprehensively as possible the data and information that is available on that specific topic, the exposure profile is a relatively brief compilation summary of only those data and pieces of information that will be used in the risk characterization phase of the assessment.

The exposure profile can include, as appropriate, a qualitative and/or quantitative evaluation of the magnitude, frequency, and patterns of exposure to a pathogen or indicator for each of the subpopulations in each of the scenario(s) of interest. The exposure profile should also identify the specific assumptions that are made during the analysis phase and uncertainties that are thought to be important for the risk assessment. Typically, assumptions made during the analysis phase are based on scientific judgment. A description of the uncertainty associated with each element of the exposure assessment should be provided to the extent that it is reasonable and possible.

The description of the assumptions and uncertainties related to exposure should provide insight into the strengths and weaknesses of the assessment for evaluation during risk characterization. For example, Teunis and Havelaar (1999) used the exposure profile section of their *Cryptosporidium* in drinking water risk assessment to summarize the quantitative information on concentration of oocysts in raw water, recovery of the detection method, reduction by treatment, and amount of finished water that is consumed. The distribution type (e.g., negative binomial, beta, 2 choice binomial, log-normal) selected for each parameter as well as median and 95% range are presented in a table. A description of the Monte Carlo calculations and graphical as well as narrative discussion of the Monte Carlo simulation is also included. Important observations about the results are highlighted, such as the following:

- Correction of oocysts counts for viability has little effect on the distribution of the concentration of oocysts in river water.
- The two distributions for river water and storage overlap, so that occasionally the treatment plant will be confronted with relatively high oocysts loads, even after passage of three reservoirs in series.
- Although treatment (physico-chemical) has a marked effect on oocysts concentrations (frequency distribution shifted by 4 logs), there is still a small probability of high concentrations of oocysts in treated water that is related to occasional reduced performance of the treatment plant.

As another example, Soller et al. (1999) used the exposure profile section of their rotavirus in drinking water risk assessment to summarize the exposure parameter assumptions. Below is a summary of some example assumptions that were used in that MRA:

- The exposure model assumes that there is no upstream contamination or upstream contamination has been diluted to the point that the effects are negligible.
- The exposure model assumes that there are no animal (agricultural and grazing) sources of human infectious rotavirus.
- Acute-phase infected humans engaged in water recreation near the drinking water intake could be a significant source of rotavirus. However, this was not considered significant because site specific data that would be required to add this parameter is not available and body contact water recreation is likely to be insignificant during winter months, which is the time of year when rotavirus infections are most significant.
- Wastewater treatment plant effluent is the most important source of rotavirus and is assumed to have undergone secondary treatment with chlorine, contribute 5% of river volume, and contain 1 to 375 focus forming units (ffu)/L.
- Rotavirus decay in source water results in 99% reduction between 3 and 30 days.
- Chlorine residual provides between 0 and 1.0 log reduction in rotavirus between the drinking water treatment facility and the tap.

Thus, the exposure profile serves as the critical linkage from the exposure component of the analysis phase of the microbial risk assessment to the health effects component of the analysis phase and the risk characterization phase. Moreover, consistent with the recommendations from the EPA/ILSI framework regarding the iterative and fluid nature of risk assessment, the exposure profile (as well as the host pathogen profile described in section 4.5) should be critically evaluated by the risk assessors and managers to determine if the problem formulation component needs to be revisited and refined based on the availability of relevant data presented in the exposure profile (as was illustrated graphically by the vertical arrows in Figure 3). Although the quantity and quality of data that will be available for any particular risk assessment will necessarily vary, the output from the exposure profile serves as input to the human health effects component of the exposure assessment and/or the risk characterization component of the microbial risk assessment.

4. Human Health Effects

Characterization of human health effects involves the interactive evaluation of three components of the analysis phase—host characterization, evaluation of human health effects, and quantification of the dose-response relationship (see Figure 7). These three sections are used to develop the host-pathogen profile that provides qualitative and or quantitative descriptions of the nature of the illness and quantitative dose-response analyses for the scenario(s) developed during problem formulation.

4.1 Introduction to Human Health Effects

The EPA/ILSI Framework (ILSI, 2000) identifies health effects elements that should be considered during risk assessment. Several of the most important elements are summarized below and discussed in the following sections, including:

- duration of illness;
- severity of illness;
- morbidity, mortality, sequelae (long-term effects) of illness;
- extent or amount of secondary transmission; and
- quality of life.

4.1.1 Duration of Illness

Duration of infectious disease illness is usually expressed in days. Duration can often be divided into duration of incubation (incubation period), duration of infection, duration of infectiousness (duration that host excretes the pathogen), and duration of disease symptoms. The scope of the risk assessment will determine the extent to which detailed information is required for each of these factors. If secondary transmission is expected to be significant, then incubation period and duration of infectiousness may be important determinants of the magnitude of disease occurrence.

The incubation period for a disease is the interval from a person's exposure to the pathogen to the time they develop symptoms or clinical illness (or the period between the dose and some measurable response, such as shedding of the pathogen or serological response). Different diseases have different incubation periods, and this information can be used to help identify the pathogen responsible for a particular outbreak. Chronic sequelae from infections include all persistent and future effects on health (disability, recurrence of infection) and may extend for years after acute infection (see more below). A brief summary of some incubation periods for several waterborne diseases is presented in Table 3.

Table 3. Typical Incubation Periods for Some Waterborne Pathogens

| Pathogen | Incubation Period ^a |
|---------------------------------|---|
| <i>Cryptosporidium parvum</i> | 2-10 days (average 7 days) ²² |
| <i>Giardia lamblia</i> | 1-2 weeks (average 7 days) ²³ |
| <i>Shigella</i> spp. | 16-72 hours ²⁴ |
| <i>Campylobacter jejuni</i> | 2-5 days (Trachoo, 2003) |
| <i>Escherichia coli</i> O157:H7 | 1-8 days (average 3-4 days) (Weir, 2000) |
| Rotaviruses | < 4 days (average 1-2 days) (Aitken and Jeffries, 2001) |

^a Defined as time from exposure to onset of first symptoms.

4.1.2 Severity of Illness

The severity of illness, morbidity, mortality, and chronic sequelae of illness are all factors that need to be considered in the choice of health endpoints considered in the risk assessment. Severity of illness is often hard to quantify because disease symptoms often include subjective descriptions. Severity of illness can also be measured by more objective parameters, such as T-cell count or other biological markers (e.g., liver function). Number of physician visits, hospitalizations, or emergency room visits may also be used to assess severity, but these measures have the disadvantage that they depend on the availability of such services, cultural and social values related to the use of medical services, and costs. Severity of symptoms may be related to whether an individual has a naïve immune system with respect to the pathogen or if the individual has partial immunity. Severity of infection does not necessarily equate to severity of illness. A severe infection, where pathogens are multiplying in the host in great numbers, may not be accompanied by severe symptoms or any symptoms that the infected individual can notice. Individuals that are infected and are able to transmit the disease, but do not exhibit symptoms, are known as carriers. The length of time an individual remains in the carrier state can vary based on pathogen and host factors. Severity of illness and severity of infection are usually used in reference to an individual (as opposed to a population). An individual may also exhibit varying degree of infectiousness during the course of an infection and infectiousness between individuals can be different. Severe illness may or may not be accompanied by severe infectiousness.

4.1.3 Morbidity, Mortality, and Sequelae

Morbidity and mortality measures can also be used to characterize disease burdens within a population. Morbidity is a measure of the number of people who are afflicted with a given disease or who display a given symptom per unit of population (e.g., per 1000 people, per 100,000 people). Mortality is a measure of the number of deaths per unit population, or number

²² http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_cryptosporidiosis.htm

²³ <http://www.cdc.gov/ncidod/dpd/parasites/giardiasis/default.htm>

²⁴ <http://pathport.vbi.vt.edu/pathinfo/pathogens/Shigella.html>

of deaths out of the diseased population. Both morbidity and mortality are most commonly expressed as annual rates (or rates during an outbreak). Disability-adjusted life years (DALYs), which measure and compare the impacts of disease burden on a population, use morbidity (years lived with a disability), mortality (years of lost life), and standardized life expectancy to calculate a DALY value for a given disease for a defined population. Prüss et al. (2002) employed a scenario-based approach to estimate the burden of disease (in DALYs) at national and sub-national levels (divided global population in to 14 geographical regions) for water-related exposures, sanitation, hygiene, and behaviors. They estimated the disease burden from water, sanitation, and hygiene to be 4.0% of all deaths and 5.7% of the total disease burden (in DALYs) occurring worldwide, taking into account diarrheal diseases, schistosomiasis, trachoma, ascariasis, trichuriasis, and hookworm disease.

Sequelae of illness, which are more commonly referred to as “chronic sequelae,” are conditions that occur after infection has occurred. Because chronic symptoms may be removed in time from the acute infection, it is often harder to demonstrate a correlation between infection and symptoms. Furthermore, the type of epidemiological study design that could detect chronic sequelae (i.e., retrospective cohort study design) is not commonly conducted for waterborne illnesses. Some well-known examples of chronic sequelae for pathogens (and a toxin) include, but are not limited to, the following (Amvrosieva et al., 2001; Begier et al., 2008; Carbone et al., 2005; Gerba, 2006; Girschick et al., 2008; Haas et al., 1999; Hauri et al., 2004; Jaidane and Hober, 2008):²⁵

- auto immune disease, such as reactive arthritis (associated with *Chlamydia*, *Salmonella*, *Shigella*, *Yersinia*, *Brucella*, *Leptospira*, *Mycobacteria*, *Neisseria*, *Staphylococcus*, and *Streptococcus*);
- Guillian Barré syndrome (associated with *Campylobacter*);
- hemolytic-uremic syndrome, renal failure (associated with *E. coli* O157:H7);
- Type 1 diabetes (initiated or accelerated by Coxsackievirus B4);
- ulcers and stomach cancer (associated with *Helicobacter*);
- failure to thrive, lactose intolerance, chronic joint pain (associated with *Giardia*); and
- neurological effects (associated with poliovirus, echovirus, Coxsackievirus, *Listeria*, botulinum toxin).

4.1.4 Secondary Transmission

As noted previously, secondary transmission refers to infection spreading from one infected person to another susceptible person. For an introduction to secondary transmission refer to Section 2.3.1. Specific examples of secondary transmission modeling in microbial risk assessment are provided by Soller (2008) who evaluated the potential impacts of secondary transmission on the Ground Water Rule, and Chick et al. (2001) who evaluated the transmission of infection using *Cryptosporidium* in a case study.

²⁵ <http://www.clevelandclinicmeded.com/diseasemanagement/infectiousdisease/foodborne/table2.htm>

4.1.5 Quality of Life

Quality of life captures the impacts that illness has other than medical costs and lost work hours. It is particularly relevant for chronic illnesses that cause pain, suffering, and a sacrifice in lifestyle. One concept, known as quality-adjusted life years (QALY), is a method for assigning a numerical value for quality of life and translating that numerical value to a monetary measure (WHO, 2001). Duration and severity of illness can also be used to characterize quality of life, but these are not expressed in monetary units, so would not be utilized in the same manner as QALYs. DALYs are recommended in WHO *Water Quality: Guidelines, Standards and Health* to integrate the effects of a single agent, compare the health effects of different agents or conditions, and to inform the debate on acceptable risk (WHO, 2001). WHO expects that “DALYs will play an important role in prioritizing risk factors, determining levels of acceptable risk, setting health targets and appraising effectiveness [of policy or mitigation] through examining public health outcome.” DALYs and QALYs are not calculated in the same manner and have reversed scales of measure. DALYs measure a health gap, with full health represented as 0 and full disability (death) as 1.0; QALYs measure health expectancy, with full health represented as 1.0 and lowest possible health state (death) as 0 (Airoidi and Morton, 2009; Gold et al., 2002; Rice et al., 2006).

It is important to note that QALYs and DALYs are not objective measures and require a descriptive conceptualization of health states. In addition, there can be significant differences in ranking due to ethnicity, gender, and area of residence (different cities; urban versus rural). Thus, there is much controversy regarding the validity of these measures partially because there is no accepted “gold standard” for determining criterion validity (Gold et al., 2002).

EPA has used QALYs and Morbidity Inclusive Life Years (MILYs)²⁶ in the regulatory impact analysis for the Final Clean Air Interstate Rule (EPA, 2005c, CAIR Appendix G) and the Long Term 2 Enhanced Surface Water Treatment Rule. (EPA, 2006a, LT2 Appendix U). These types of measures are usually included in cost effectiveness analyses (CEA) rather than within risk assessment. Risk assessors should be aware of how the risk assessment results might be used, such as in a CEA.

4.2 Dose-Response Analysis Overview

Risk assessment is the process by which information on exposure, toxicity or infectivity, and the characteristics of the exposed population, are combined to estimate the probability and severity of harm from the agent in question. In the case of waterborne microbial contaminants, risk assessment generally involves estimating the probability of illness or infection based on exposure or intake estimates and exposure-response or dose-response relationships.

During dose-response analysis, data from human clinical studies, epidemiological studies, animal studies, and/or outbreaks are used to develop a mathematical relationship between the intensity

²⁶ MILY combines QALYs saved from avoided cases of non-fatal morbidity with life years resulting from mortality risk reductions (assigned a weight of 1.0)

of exposure or amount of intake and the subsequent occurrence of disease or infection. Dose-response models are generally derived using statistical estimation techniques, and the form of the relationship between exposure and response is determined by (1) assumptions related to the biological processes leading to infection, and (2) the “shape” of the relationship found in the data between exposure and the health outcome of interest.

A number of factors need to be addressed in the derivation of dose-response models and estimation of their parameters for microbial risk assessment, including the following (adapted from ILSI, 2000):

Dose-response factors

- statistical model(s) to analyze or quantify dose-response relationships;
- human dose-response data;
- animal dose-response data; and
- source and/or preparation of challenge material or inoculums.

Factors that overlap with exposure analysis

- utilization of outbreak or intervention data (could be used to build exposure scenarios);
- route of exposure or administration; and
- duration and multiplicity of exposure.

Factors that overlap with health effects

- characteristics of the exposed population (age, immune status, etc.); and
- organism type and strain (including virulence factors or other measures of pathogenicity).

The mathematical form of the dose-response model may vary with pathogen or strain, route of administration, distribution of host statuses, and other factors. An overview of common dose-response models for microbial based infections is provided below. Either human or animal data may be used to derive dose-response estimates—though human data are generally preferred—and occasionally information from studies of disease outbreaks may provide useful information about both primary infection risks (infections arising directly from exposure) and secondary transmission (person-to-person).

Knowledge of the conditions under which dose-response data were collected is essential both for those developing dose-response models and for those evaluating dose-response models for use in MRA. In particular, the strain of the pathogen, animal model, and route of inoculation can strongly influence the dose-response. Thus, extrapolation of such dose-response relationships to conditions other than those for which data were collected should be done only in conjunction with justification and with a full description of the conditions for which the dose-response model was developed. For example, dose-response models have been proposed based on data collected during experiments with animal hosts whose response appears to differ substantially from that of humans. Comparison of murine response to *Listeria monocytogenes* (based on feeding studies) to that of humans (based on epidemiological data) indicates a factor of $\sim 10^6$ difference in LD₅₀

between the two hosts (FDA/USDA, 2003). Numerous studies of pathogen-host combinations (e.g., Bell et al., 1955, for tularemia; Holdenfried and Quan, 1956, for plague) have shown the potential for wide variation in response between animal hosts and even among animal hosts of the same species but of different geographic origin. Insights into the applicability of animals as models of human infection may sometimes be drawn from pathology literature; in many cases animal models are selected for pathology experiments based on similarities that their infection process bears to that of humans (Lyons and Wu, 2007). Knowing and reporting these similarities may provide information for interpretation of dose-response data. Likewise, because dose-response is dependent upon inoculation route (e.g., Couch et al., 1966; Quan et al., 1956), dose-response models should be chosen for incorporation into quantitative microbial risk assessments (QMRA) that are consistent with the relevant exposure route.

Some key factors that influence the utility of a dose-response study, because they impact infectivity of a pathogen and host response, are route of exposure or administration (e.g., oral, dermal, subcutaneous) and the source and preparation of the challenge material or inoculum. Different strains of a pathogen may have very different degrees of infectivity and the strain or strains used to generate the experimental data may or may not be the most relevant to the population that is exposed in the exposure scenario that is being modeled. Furthermore, and as noted previously, susceptibility in the general population may vary greatly with age, general immune status, and pre-existing or acquired immunity to specific pathogens and pathogen strains. The narrative accompanying the modeling should explain the potential impacts of different strains (or other sub-classifications of pathogens such as serovar or single nucleotide polymorphisms) on the outcome of the risk calculation. The potential uncertainty associated with variation in infectivity should be calculated quantitatively if possible. Although most MRAs consider severity of symptoms to be independent of dose size, there is some evidence for dose-dependence of severity of symptoms (Text Box 7).

The duration of exposure, the number of exposures, and time between exposures may affect the probability of an adverse health effect, as estimated by the dose-response relationship. As discussed previously, determining the independence or lack of independence of exposure events is complicated by host status as well as pathogen characteristics. For example, individuals who are already infected with a particular pathogen should not be considered susceptible to reinfection by the same pathogen while infected. Previously infected but recovered individuals have decreasing immunity over time. However, the nature of the decrease in immunity depends on many conditions including whether subsequent exposures, which did not result in infection boost immunity, host factors that relate to overall health of the immune system, and pathogen factors such as rapidly evolving antigenic epitopes. Because any given person's immunity fluctuates based on many host factors, and because different pathogens elicit different immune responses, it is difficult to define a single exposure duration that best describes all combinations of host-pathogen interactions. Given the variability and complicated nature of capturing all appropriate exposure durations, most risk assessments choose a default exposure event duration (e.g., all water consumed in one day).

Text Box 7. Dose-Dependency of Host-Pathogen Interactions

Most MRAs assume that severity of health endpoint is not influenced by magnitude of dose. For example, with *Cryptosporidium*, whether an individual is exposed to 1 or 10,000 organisms, if they become infected and ill the health end points are similar in severity. Thus, the assumption is made that exposure to a larger *Cryptosporidium* dose will not result in worse symptoms (EPA, 2006a). Although there is emerging evidence that this assumption may not be appropriate for all pathogens at all doses, the data is generally insufficient to be included in quantitative risk assessment. However, if there is evidence of dose-dependent severity of symptoms for the pathogen of interest, then it should be discussed. For example, pathogenic *E. coli* feeding studies in human volunteers demonstrated dose-dependency of disease severity and suggested that volume of liquid stool can be used as a quantitative metric for illness severity (Bierber et al., 1998). Colwell et al. (2003) reported that in Bangladeshi villages where sari or nylon cloth was used to filter surface water, the number of cholera cases was reduced and the severity of disease was reduced compared to villages that did not filter their surface water. Nauta et al., (2009) compared six *Campylobacter* risk assessments and concluded that the most effective public health intervention measures targeted *Campylobacter* concentration reductions, rather than reducing its prevalence.

4.3 Overview of Common Dose-Response Model Forms for Pathogens

This section provides a brief summary of and introduction to the most commonly used dose-response models for microbial pathogens. Namata et al. (2008) also provides a useful summary of dose-response models for MRA. Although providing state-of-the-art guidance on deriving dose-response relationships is beyond the scope of this MRA Protocol, there are several issues that risk analysts and risk managers should be aware of when evaluating the dose-response literature. [Appendix G](#) provides additional information on dose-response modeling and includes discussions on the following topics:

- choosing a model for microbial dose-response;
- threshold assumptions;
- sources of uncertainty in dose-response models; and
- sources of dose-response data.

The objective of the dose-response assessment is to develop a relationship between the number of microbes a person or population has been exposed to and the likelihood of occurrence of an adverse consequence (health outcome). In general, dose-response analysis would be relatively straightforward if the level of microbial risk that was deemed acceptable were sufficiently high to allow experimentation that would permit the direct assessment of risk in the observable range (Haas et al., 1999). However, the probability of infection (risk) from a single low-dose exposure event is often sufficiently low that use of direct observation (or experimentation) is impractical. Thus, the use of parametric dose-response curves to facilitate extrapolation into the low-dose range that matches the risk level of concern is necessary.

Dose-response models are mathematical functions that take as input the dose to which individuals or populations are exposed and yield a probability (bounded by 0 and 1) of the particular adverse health effect (Haas et al., 1999). These dose-response functions play a prominent role in risk assessments for pathogens in water because they effectively translate exposures into risks. In real world situations where large numbers of individuals may be exposed (e.g., PWSs), relatively low individual risk levels may be of concern from a public health perspective because even low individual risks can translate into a large number of illnesses.

The two most commonly used dose-response relations are the exponential and beta-Poisson models. The exponential and beta-Poisson models are only valid, however, when their underlying assumptions are met. More computationally intensive dose-response relations are also available for conditions in which neither the exponential or beta-Poisson models are appropriate. Alternative two-parameter models have been proposed for use in microbial risk assessment, including the log-normal, log-logistic, extreme value models (Pinsky, 2000). Three-parameter models that have been suggested for MRA include the Weibull gamma (Farber et al., 1996), exponential gamma, Weibull exponential, and the shifted Weibull model (Kodell et al., 2002). Although three-parameter models are more flexible than two-parameter models, they require data at four or more doses, which is usually not available for many microbial pathogens. Research continues to be conducted on appropriate methods for selection of models from among these and other candidate models (e.g., Moon et al., 2004, 2005).

The models discussed in this section estimate risks for exposed individuals. Population-level risks (i.e., the incidence of disease among a group of exposed individuals) are generally constructed by combining individual risks with estimates of the distribution of doses to the exposed population.

To promote transparency and clarity in an MRA, the following points should be addressed for each model presented:

- Discuss assumptions inherent in making extrapolations to doses lower than those used in studies.
- Provide a detailed description of dose-response and risk assessment modeling approaches, including the applicability of the models for use in various exposure situations and for various pathogens.
- Inform risk assessment users of the models' key assumptions.
- Discuss the type of information that the various models are expected to provide.
- Discuss limitations of the models.
- Discuss the use of likelihood methods to compare how well dose-response models fit the data.
- Discuss the biological rationale for the model selected.
- Articulate strengths/weaknesses and advantages/disadvantages of the models; include a comparison of the benefits and limitations of the chosen models versus other potential models.
- Discuss flexibility in approaches to the dose-response relationship depending on the pathogen being considered and the assumption about a no-threshold effect (i.e., can it be

assumed that one organism is sufficient to produce infection in some portion of an exposed population or subgroup?).

To take biological mechanisms into account, a dose-response model for microbes should account for the heterogeneous distribution (random or clumping) of microbes in water (affecting exposure) and a microbe's ability to reproduce in the human body (linked to pathogenicity) (Haas et al., 1999). Laboratory dose-response studies are usually conducted under conditions in which the microorganisms are randomly distributed in the administered dose. This is known as a Poisson distribution. The framework for exponential models is based on well-studied mathematical relationships; however, the model parameters use empirical data from experimental and epidemiological studies that are organism specific (e.g., an organism's ID₅₀). A concern for environmental water samples regarding the Poisson distribution is clumping, association with suspended solids and other spatial distribution issues; however, this phenomenon can be accounted for in dose-response modeling (see Teunis and Havelaar, 2000, for further information).

The dose-response relationship that is defined by the equation is "fit" to experimental data using a variety of statistical methods. If the model is a good fit, it will predict risks that are close to those actually observed within the range of experimentally administered doses. However, the doses used in volunteer studies may be higher than those typically encountered in the environment, so it is necessary to extrapolate the risks associated with lower doses using the model derived from the higher doses. In extrapolating to lower doses, risk assessors rely on the belief that the form of the dose-response model is based on an accurate representation of the infection process that holds at low doses as well as high doses. Text Boxes 8 and 9 illustrate how experimental data on Noroviruses and *Cryptosporidium* have been used to derive dose-response relationships for these pathogens. Recently, outbreak data have been used to derive dose-response relationships for several pathogens. Teunis et al. (2004, 2008a) and Bollaerts et al. (2008) provide good examples of how outbreak data can be used to derive dose-response relationships.

Several published studies (e.g., Coleman and Marks, 2000; Nauta et al., 2009) suggest that extrapolation of these dose-response models in most common use for infection and illness endpoints for waterborne exposures may not be advisable, given the complexity of the pathology of illnesses and given the relatively low reported incidence of illness and the relatively high daily exposure of humans to pathogens (Levin and Antia, 2001). Although a critical evaluation of this perspective is difficult to provide, given the limited data available for human response to exposure to pathogens of known dose and characteristics, mechanistic modeling (described in Appendix G) offers an avenue for development of improved models for extrapolation to low-dose.

4.3.1 Exponential Model

The exponential model is the simplest model that is commonly used in MRA; it is based on the following assumptions (Haas et al., 1999):

Text Box 8. Brief Summary of Challenge Studies to Investigate the Dose-Response and Host-Immunity Factors Related to Norovirus Infection

The group of viruses called norovirus (previously known as Norwalk and Norwalk-like viruses) is the most common cause of AGI outbreaks (e.g., through ingestion of contaminated food and water) in the United States. Reports implicated noroviruses in 94% of U.S. nonbacterial gastroenteritis outbreaks from 1996-1997 (Fankhauser et al., 1998). However, to date, host immunity to noroviruses has been poorly characterized. Although over 70% of U.S. adults have serum antibodies to Norwalk virus by middle age, those antibodies do not appear to confer any protection from reinfection (Greenberg et al., 1979); thus, it appears that people are likely to be repeatedly infected throughout their lifetimes. Despite outbreak studies suggesting that norovirus has high infectivity and high person-to-person transmissibility, certain exposed people never develop illness (i.e., remain asymptomatic).

Lindesmith and colleagues (2003, 2005) conducted a series of human volunteer studies to examine the dose-response characteristics of different strains of noroviruses (Norwalk [NV] and Snow Mountain Agent Virus [SMV]) and the role of host immunity in the probability of (re)infection. For these studies, the researchers recruited healthy adult volunteers with and without pre-existing serum IgG to noroviruses. The first study included 31 volunteers and examined 3 low doses of NV. The second study included 15 volunteers and examined 3 doses of SMV. The volunteers drank sodium bicarbonate solution before and after the challenge inoculum to neutralize stomach acidity. The inoculum was diluted in sterile water and ingested. The volunteers stayed 5 consecutive days/6 consecutive nights at a research center for monitoring of GI symptoms, then reported for follow-up visits for collection of stool, serum, and saliva samples on days 8, 14, and 21 post-challenge.

Infection was defined as detection of viral shedding in stool by reverse transcriptase polymerase chain reaction (RT-PCR) or seroconversion designated by a four-fold or more rise in the specific IgG. Symptoms that defined illness were diarrhea (defined as more than 2 unformed stools within 24 hours), vomiting, abdominal pain, muscle pain, fatigue, and chills—but fever and headache were excluded.

Previous research has reported an association of a mutation in the alpha (1,2) fucosyltransferase gene (FUT2) gene with immunity to NV infection (Marionneau et al., 2002). In that study, volunteers with the FUT2 mutation remained healthy and had no significant increase in anti-Norwalk virus salivary antibody titers, even after high-dose exposure. Note that about 20% of the North American population has the FUT2 mutation. Of the volunteers with fully functioning FUT2 genes, about half became infected. In the remaining uninfected half of the group, salivary IgA levels showed mucosal immune response post-challenge, suggesting that previous exposure had resulted in protective immunity.

Moe et al. (2002) found that infected subjects were generally older than uninfected subjects and were twice as likely to have NV-specific IgG in their baseline serum specimen. Consequently, the presence of anti-NV serum IgG was not protective against infection. In other words, although these individuals had been exposed previously to NV, perhaps multiple times, they continued to be susceptible to reinfection. These studies provide important implications for microbial risk assessors—even with a very low infectious dose in susceptible populations (<1 PCR detectable unit), susceptibility to Norwalk virus is multifactorial and influenced by both acquired immunity and genetic traits. Additional studies are planned and underway to assess whether other strains of norovirus have similar host-susceptibility factors.

In subsequent work, these researchers along with Teunis et al. (2008b), developed a dose-response relationship for NV based on challenge study data and a new variant on the hit theory model of microbial infection. This relationship accounts for variation in NV infectivity, as well as the degree of virus aggregation. The results indicate that passage through a human host does not change NV infectivity and that the average probability of infection for a single NV particle is close to 0.5.

Text Box 9. Brief Summary of *Cryptosporidium* Feeding Studies

Human feeding studies have been used for decades to systematically evaluate dose-response effects for pathogens. Chappell and colleagues (Chappell et al., 1999; DuPont et al., 1995; Okhuysen et al., 1999) have conducted volunteer feeding studies using three strains of *Cryptosporidium parvum* that have formed the basis of dose-response parameters used in several MRAs. The following is a brief summary of the methodology involved with creating dose-response data sets using volunteers, which shows the time and resource-intensity of such studies.

Students and employees of the University of Texas Health Science Center and others in the surrounding area of Houston were recruited as volunteers. Volunteers could not be caretakers of infants, elderly, or those with chronic diseases or immunosuppression. Recruits were given extensive information on cryptosporidiosis and had to score 100% on a written examination that tested the recruits on their comprehension of the study, the fact that they could become ill, that there was no effective treatment for the illness, and that the organisms could be spread to household contacts. The next stage of their evaluation for inclusion in the study involved providing medical histories and passing extensive medical tests.

In each of the three studies, the volunteers ingested a single known dose of viable *C. parvum* oocysts of one of three isolates: IOWA, TAMU, and UCP. The oocysts were counted multiple times with a hemacytometer and delivered to an empty stomach in gelatin capsules washed down with 250 mL of saline. The subjects were given anywhere from 10 to 1,000,000 oocysts per dose.

Volunteers submitted stools passed after the challenge and completed daily diaries regarding their stool passage and any symptoms; household contacts were also monitored for diarrheal illness. Volunteers received oral electrolyte solution to treat any diarrheal episodes. Blood was collected from each of the volunteers at specified days post-challenge and tested for antibody response.

To measure the challenge responses, the study authors considered two definitions of infection—confirmed and presumed. A confirmed infection was based on oocysts detected in stools using direct fluorescence assay. Some volunteers who had oocysts in their stools did not develop any symptoms. On the other hand, some volunteers had illness symptoms that were indistinguishable from those with confirmed infection, but had no detectable oocysts in their stools. Because of the detection limit of the assay methodology, these volunteers were presumed to be infected.

As part of the response assessment, the classification of GI symptoms was established before the study; symptomatic individuals were defined as having two or more concurrent gastrointestinal complaints. Diarrhea was defined as the production of 200 grams or more of unformed stool per day, 3 or more unformed stools in 8 hours, or 4 or more unformed stools in 24 hours. Those volunteers who did not have either GI symptoms or fecal oocysts throughout the study were presumed to be uninfected.

Because the purpose of the studies was to develop dose-response curves for the different strains based on infectious dose, it was important to be able to capture data on the median infectious dose. The first study of the IOWA isolate was designed to cover a wide range of doses (30-1,000,000 oocysts) so to more effectively capture the median dose. The doses of the other two isolates were adapted as the study progressed to narrow the range of doses; that is, the first group of volunteers were challenged at a moderate dose, while the next group's dose level was altered, depending on the outcome of the previous group. That way, the median infectious doses could be captured over a smaller dose range. The entire time required for each dose-response study was 11 to 14 months.

The infectivity for each of the three isolates was estimated using the study data and then tested using the exponential model (Messner et al., 2001). A comprehensive dose-response evaluation was conducted by EPA during the development of the LT2 (EPA, 2003a,b, 2006a).

- microorganisms are distributed in water randomly²⁷ and thus, follow the Poisson distribution;
- for infection to occur, at least one pathogen entity must survive within the host; and
- the probability of infection (in a person or animal model) per ingested or inhaled organism is constant.²⁸

Under the exponential model, there is no minimum infectious dose, as a nonzero risk is predicted with any non-zero dose. Assuming that a single organism is sufficient to cause infection, and that the ingested organisms must pass through “multiple barriers” to survive long enough to cause disease, yields the exponential risk model:

$$P_r = 1 - e^{-rD}, \quad [4-1]$$

Where:

P_r is the probability of an individual exhibiting the response specified by dose-response parameter given exposure to the defined dose (unitless);

D is the ingested dose of microbes (number of microbes ingested per event, which is often represented as daily water intake times concentration);

r is the dose-response parameter that is “fit” to the data; higher value indicates higher risks at lower doses²⁹; and

e is the base of the natural logarithm function (unitless).³⁰

The “response” to exposure may be the development of clinical symptoms, and/or microbiological or immunological evidence that microbes have persisted or multiplied in the body. For example, shedding of oocysts (regardless of whether illness symptoms are present) in stool is commonly used to indicate *Cryptosporidium* infection.

Under this model the median infectious dose is $N_{50} = 0.693/r$.

Text Box 10 illustrates the use of the exponential model in microbial risk assessment.

4.3.2 Beta-Poisson Model

The beta-Poisson model is based on similar assumptions to the exponential model except that the third assumption (that the probability of infection per ingested organism is constant) is relaxed. This model allows the probability of infection per ingested or inhaled organism to vary within the exposed population (Haas et al., 1999). In this model the probability of surviving and reaching a host site (r in the exponential model) is beta distributed, and thus the model contains the two parameters (α and β) of the beta distribution. The exponential model generally provides a good fit to experimental data if the infectivity of the administered organisms and the inherent susceptibility of the exposed population (animal or human) are constant. However, when there is variability in the host-pathogen interaction, diversity in the pathogen (as when multiple strains

²⁷ As noted previously, because microbes are generally not thought to be distributed randomly in environmental media, this assumption is considered to be a limitation of the exponential model unless adjustments are made as discussed in Teunis and Havelaar (2000).

²⁸ This assumption also introduces uncertainty because host variation is not considered.

²⁹ For small values of r , the estimated individual risk is $r \times d$ (i.e., the model is linear at low doses).

³⁰ Euler’s number or Napier’s constant is ~ 2.71828 .

Text Box 10. Summary of Use of Exponential Model

(Source: Rose et al., 1991)

Rose and colleagues (1991) used an exponential model to estimate the risk of infection after exposure to treated water contaminated with *Giardia* cysts as shown by the following equation:

$$P_i = 1 - \exp(-r\mu V),$$

where P_i is the probability of infection, r is the host-pathogen interaction probability, μ is the average number of organisms, and V is the volume of water consumed.

The parameter designating the infectivity of *Giardia* (r) in the exponential model was based on data from studies in which volunteers were fed a range of 1 to 10^6 cysts and the response was measured by the number of cysts excreted in the volunteer feces, not by clinical symptoms (Rendtorff, 1954; Rendtorff and Holt, 1954). An average r value (the fraction of microorganisms that are ingested that survive to initiate infection) was compared by determining the value of r at each dose. Based on the results, the average r was calculated to be 0.01982.

Using the exponential model, the potential risk of infection was determined with varying levels of *Giardia* cysts in drinking water. The model used two liters of water a day as the consumption parameter, V . The number of cysts (μ) was based on concentrations measured in source waters with 99.9, 99.99, and 99.999% estimated removal by treatment. The exposure was based on the numbers of cysts per liter multiplied by 2L. A maximum daily risk was estimated using the highest level of contamination and a yearly risk was based on 365 days of exposure to the geometric mean concentration of cysts. The model was checked for plausibility by entering the data from five waterborne giardiasis outbreaks using the levels of *Giardia* cysts and the observed attack rates in the exposed population.

are present), or both, the dose-response relation tends to be shallower than that of the exponential relation. The most commonly used approximation to the beta-Poisson model is as follows:

$$P_r = 1 - (1 + D/\beta)^{-\alpha} \quad [4-2]$$

Where:

P_r is the probability of an individual exhibiting the response specified by dose-response parameter given exposure to the defined dose (unitless);

D is the ingested dose of microbes (number of microbes ingested per event, which is often represented as daily water intake times concentration);

β is the location parameter; determines inflection point of dose-response curve (unitless); and

α is the shape parameter governing the steepness of the dose-response curve (unitless).

Unfortunately, in this approximation to the beta-Poisson model, α does not have an obvious physical interpretation. What can be said is that it is a shape parameter governing the steepness of the dose-response curve; the larger its value the steeper the curve (McBride et al., 2002). The derivation of the approximation to the beta-Poisson model, as shown above requires that $\beta \gg 1$, $\beta \gg \alpha$, and becomes a poorer approximation at small values of β or large values of D . In practice, this condition is not always met and caution is warranted when this approximation is used (Teunis and Havelaar, 2000). This approximation to the beta-Poisson is linear at low doses and the curve is always shallower than the exponential model. However, as α approaches ∞ , the approximate beta-Poisson model approaches the exponential model (Haas et al., 1999).

Under this model the median infectious dose is $N_{50} = \beta \times (2^{1/\alpha} - 1)$.

When possible, it is preferable to directly fit the exact beta-Poisson model (Equation 4-3), where ${}_1F_1(\alpha, \alpha + \beta, -D)$ denotes a confluent hypergeometric distribution with the specified parameters.

$$P_r = 1 - {}_1F_1(\alpha, \alpha + \beta, -D) \quad [4-3]$$

Where:

P_r is the probability of an individual exhibiting the response specified by dose-response parameter given exposure to the defined dose (unitless);

D is the ingested dose of microbes (number of microbes ingested per event, which is often represented as daily water intake times concentration); and

${}_1F_1(\alpha, \alpha + \beta, -D)$ is Kummer's confluent hypergeometric distribution, with parameters α , $\alpha + \beta$, and $-D$, variable means are analogous to the beta-Poisson approximation.

Although the hypergeometric distribution generally has no analytical solution, numerical estimation algorithms have been developed.

4.3.3 Bayesian Methods

Bayesian methods to estimate dose-response model parameters are also being increasingly (Englehardt, 2004; Englehardt and Swartout, 2004; Messner et al., 2001). In general, a dose-response function gives the probability of illness or infection as a function of the dose and of several unknown parameters. Experimental data are collected from subjects accidentally or deliberately exposed to a measured microbial dose. The numbers of subjects that become infected or ill for each dose level are observed, leading to a binomial likelihood, which is the probability of the observed numbers of cases given the unknown parameters. The “traditional” frequentist statistical approach uses the binomial likelihood, and chooses parameter values to maximize the likelihood. Uncertainty intervals for the parameters are called confidence intervals (i.e., on average, out of 100 95% confidence intervals, 95 will contain the parameter value). Confidence intervals around the maximum likelihood estimates can be calculated using approximations valid for large samples. For small sample sizes that are typical in MRA, bootstrap confidence intervals are calculated by randomly resampling from the original dose-response data and estimating the parameters for each of these bootstrap samples.

Bayesian methods, which are discussed in more detail in [Appendix G](#), exploit available subjective and related information in addition to the numeric data. Ideally, the investigator expresses an initial assessment of the unknown parameter distribution, prior to examining the data, by defining a prior probability distribution for the parameters. The prior probability distribution is defined based on subjective information and professional judgment. Recent published MRAs have used a “non-informative” prior distribution to represent the lack of prior information. Using Bayes' rule, the posterior probability distribution for the parameters given the data can be calculated. In a Bayesian analysis, uncertainty intervals for the parameters and the dose-response function can be calculated from the posterior distribution as “credible intervals”; for example, a 95% credible interval has a 95% probability of including the parameter value, given the data.

The Markov Chain Monte Carlo (MCMC) method is often used to simulate values from a posterior probability distribution for which direct analytical calculations are difficult, intractable, or inconvenient. Gilks et al. (1996) provides a good description of these methods. Instead of being statistically independent, the consecutive values form a Markov Chain so that the statistical distribution for one value depends upon the previous value. After a sufficiently long “burn-in” period, every k^{th} value is sampled, giving an approximately random sample from the posterior distribution. It is unnecessary to know the normalizing constant that makes the distribution integrate to one. A version of the Metropolis-Hastings algorithm (Gilks and Wild, 1992; Gilks et al., 1996; Hastings, 1970) is used at each step to simulate from the posterior distribution without knowing the normalizing constant.

An advantage of the Bayesian approach over the frequentist approach is the ability to incorporate prior information. However, for the MRAs in the current literature this is not very useful because the prior information is too limited and non-informative priors have been used. The subjective nature of the choice of prior distribution is often thought to be a disadvantage of the Bayesian approach. A more important advantage of the Bayesian approach is that, unlike frequentist confidence intervals, the uncertainty intervals from a Bayesian analysis are easier to interpret and are usually interpreted correctly. Furthermore, the Bayesian uncertainty estimates of dose-response functions are generally easier to calculate and more exact than the frequentist confidence intervals. Finally, Bayesian methods are well-suited to meta-analysis of multiple studies, pathogens, or populations.

A predictive Bayesian dose-response function can be developed as follows. First, the parametric form of the dose-response function is established by theoretical derivation and, if possible, empirical confirmation. Then all available knowledge, other than the theoretical form of the conditional distribution and empirical data already used for that purpose, is considered during estimation of the parameters of the distribution. To do this, the parameters are recognized as uncertain but subject to professional judgment, and thus, a prior probability distribution is assigned to each parameter. Prior distributions are then refined with dose-response data, to obtain a posterior distribution. Next, the predictive Bayesian dose-response function can be found by multiplying the posterior by the conditional dose-response function and integrating over the parameter space (Englehardt, 2004). As noted previously, MCMC methods can then be used to generate samples from the joint posterior distribution (Messner et al., 2001).

Several researchers advocate the combined use of Bayesian and frequentist (likelihood-based) methods (Messner et al., 2001; Teunis and Havelaar, 2000). Often the frequentist approach is used to provide maximum likelihood estimates of the dose-response function and the Bayesian approach is used to calculate uncertainty intervals (e.g., 80 or 95% credible intervals for the parameters or the dose-response). Several papers use the Bayesian posterior mode to select the dose-response function (Teunis et al., 2004, 2005, 2008a,b). The posterior mode is given by the parameters that maximize the posterior probability, defined as the product of the prior and the likelihood; thus, it is not necessary to calculate the normalizing constant for this calculation.

4.3.4 Other Dose-Response Methods

In addition to the dose response models described above, there are alternative dose-response

models either in use in QMRAs or with potential for incorporation into QMRAs. These models include empirical dose response models, threshold models, and mechanistic models of varying resolution. Given the widespread use of the exponential and beta-Poisson dose-response models for waterborne pathogens and the advantages these models offer (as described above), these alternative models are not presented in the body of this report, but are described and contrasted with the exponential and beta-Poisson model in [Appendix G](#).

In brief, alternative models include empirical models (used widely in QMRAs of foodborne pathogens), threshold models, and mechanistic models. Here, the exponential and beta-Poisson models are distinguished from empirical models because their derivation is based on a sequence of plausible events, though this assessment is not universal (see, for example, Coleman and Marks, 1998). Threshold models have not been demonstrated to provide significant improvements in fit over the exponential and beta-Poisson models, but their use has been advocated on the basis of analysis of the infection process and interpretation of epidemiological data. Mechanistic models are currently in development and offer the potential for development of dose-response models for pathogens for which dose-response data are unavailable or for the low-dose range. These models depict the pathogen-host system in varying resolutions and may be stochastic, deterministic, or a combination ([Appendix G](#)).

4.4 Summary of Available Dose-Response Relationships for Waterborne Pathogens

An overview of representative dose-response relationships for waterborne pathogens is summarized in Table 4, which includes the pathogens evaluated alphabetically, the resulting dose-response form and parameter values, and the corresponding reference for that work.

4.5 Host-Pathogen Profile

The host-pathogen profile is a distillation of the most important information and data that is developed during the human health component of the analysis phase. This profile should tie together the health effects and dose-response analysis. Whereas each of the components of the human health analysis (duration of illness, severity of illness, morbidity, mortality, sequelae of illness, secondary transmission, and dose-response evaluation), describe as comprehensively as possible the data and information that is available on that specific topic, the host-pathogen profile is a relatively brief compilation summary of only those data and pieces of information that will be used in the risk characterization phase of the assessment.

The host-pathogen profile can provide, depending on the available data, a qualitative and/or quantitative description of the human health effects scenario (ILSI, 2000). An assessment of the assumptions made during the human health analysis, and the uncertainty associated with the analysis because of lack of knowledge about the scenario or insufficient experimental or epidemiological data, should be presented. Any assumptions based on scientific judgment should be described and justified in the host-pathogen profile. A summary of the quantitative or qualitative uncertainty analysis should also be included.

Thus, the host-pathogen profile serves as the critical linkage from the human health effects component of the analysis phase of the microbial risk assessment to the exposure component of

Table 4. Overview of Dose-Response Relationships for Waterborne Pathogens^a
(Source: Adapted from McBride et al., 2002)

| Microorganism | Model | Parameters ^b | Reference(s) |
|--|--|--|---|
| Adenovirus 4 | Exponential | $r = 0.4172^c$ | Crabtree et al., 1997 Haas et al., 1999 |
| <i>Campylobacter jejuni</i> ^{h,i} | Beta-Poisson | $\alpha = 0.145 \quad \beta = 7.59$ | Haas et al., 1999 Medema and Smeets, 1996 Teunis et al., 1996 |
| | Infection: Hypergeometric beta-Poisson Illness: Conditional on infection ^g | $\alpha = 0.024 \quad \beta = 0.011$ $\eta = 2.44 \times 10^8 \quad r = 3.63 \times 10^{-9}$ | Teunis et al., 2005 |
| Coxsackievirus | Exponential | $r = 0.0145$ | Haas et al., 1999 |
| <i>Cryptosporidium</i> | Exponential | $r = 0.0042$ | Haas et al., 1996, 1999 |
| | | $r = 0.077^d$ | Okhuysen et al., 1999 |
| | | $r =$ in the range 0.04 to 0.16 | EPA, 2006a |
| | Generalized beta-Poisson for Illness | $\alpha = 0.060 \quad \beta = 0.095$ | Englehardt and Swartout, 2006 |
| | Exponential | $r = 0.0128$ | Haas et al., 1999 |
| Echovirus 12 | Beta-Poisson | $\alpha = 0.401 \quad \beta = 227.2$ | Teunis et al., 1996 |
| | | $\alpha = 0.374 \quad \beta = 186.69$ | Regli et al., 1991 Rose and Sobsey, 1993 |
| | | $\alpha = 1.3 \quad \beta = 75$ | Rose and Gerba, 1991 |
| <i>Endamoeba coli</i> | Beta-Poisson | $\alpha = 0.1008 \quad \beta = 0.3522$ | Haas et al., 1999 |
| <i>Escherichia coli</i> (pathogenic strains) | Beta-Poisson | $\alpha = 0.1778 \quad \beta = 1.78 \times 10^6$ | Haas et al., 1999 |
| <i>E. coli</i> O157:H7 | Beta-Poisson ^e | $\alpha = 0.248 \quad \beta = 48.80$ | Teunis et al., 2008a |
| | Hypergeometric beta-Poisson | $\alpha = 0.084 \quad \beta = 1.44$ (children) $\alpha = 0.050 \quad \beta = 1.001$ (adults) | Teunis et al., 2004 |
| <i>Giardia lamblia</i> | Exponential | $r = 0.0199$ | Haas et al., 1999 Regli et al., 1991 Rose and Gerba, 1991 Rose et al., 1991 Teunis et al., 1996 |
| Hepatitis A virus | Exponential | $r = 0.5486^f$ | Haas et al., 1999 |
| <i>Legionella</i> | Exponential | $r = 0.06$ | Armstrong and Haas, 2008 |
| Norovirus | Infection (with aggregation): Hypergeometric function ${}_2F_1$ Illness: Conditional on Infection ^g | $\alpha = 0.040 \quad \beta = 0.055$ $a = 0.9997$ $\eta = 2.55 \times 10^{-3} \quad r = 0.086$ | Teunis et al., 2008b |
| Poliovirus I | Beta-Poisson | $\alpha = 0.1097 \quad \beta = 1524$ | Regli et al., 1991 Rose and Sobsey, 1993 |

| Microorganism | Model | Parameters ^b | Reference(s) |
|---------------------------------|--|---|--|
| | | $\alpha = 15$ $\beta = 1000$ | Rose and Gerba, 1991 |
| | Exponential | $r = 0.009102$ | Haas et al., 1999 Regli et al., 1991 Rose and Sobsey, 1993 |
| Poliovirus III | Beta-Poisson | $\alpha = 0.409$ $\beta = 0.788$ | Rose and Sobsey, 1993 |
| | | $\alpha = 0.409$ $\beta = 0.788$ | Regli et al., 1991 |
| | | $\alpha = 0.5$ $\beta = 1.14$ | Rose and Gerba, 1991 |
| Rotavirus | Beta-Poisson | $\alpha = 0.26$ $\beta = 0.42$ | Gerba et al., 1996b |
| | | $\alpha = 0.2531$ $\beta = 0.4265$ | Haas et al., 1999 Regli et al., 1991 Rose and Sobsey, 1993 |
| | | $\alpha = 0.232$ $\beta = 0.247$ | Rose and Gerba, 1991 |
| | Hypergeometric beta-Poisson | $\alpha = 0.167$ $\beta = 0.191$ | Teunis and Havelaar, 2000 |
| <i>Salmonella</i> spp. | Beta-Poisson | $\alpha = 0.33$ $\beta = 139.9$ | Rose and Gerba, 1991 |
| | Gompertz log | $\ln(a)$ in the range 29 to 50 $b = 2.148$ | Coleman and Marks, 2000 Coleman et al., 2004 Soller et al., 2007 |
| | Generalized linear mixed models and fractional polynomials of dose | $\beta_0 = 0.323$ $\beta_1 = 5.616$ $\beta_2 = -8.462$ $\beta_3 = -7.782$ $d^2 = 0.780$ | Bollaerts et al., 2008 |
| <i>Salmonella</i> (non-typhoid) | Beta-Poisson | $\alpha = 0.3126$ $\beta = 2884$ | Haas et al., 1999 |
| <i>Salmonella typhi</i> | Fractional polynomials | $\beta_1 = -18.1425$ $\beta_2 = 22.5300 \times 10^{-5}$ | Namata et al., 2008 |
| | Beta-Poisson | $\alpha = 0.1086$ $\beta = 6,097$ | Haas et al., 1999 |
| | | $\alpha = 0.21$ $\beta = 5,531$ | Rose and Gerba, 1991 |
| <i>Shigella</i> | Beta-Poisson | $\alpha = 0.21$ $\beta = 42.86$ | Haas et al., 1999 |
| <i>Vibrio cholera</i> | Beta-Poisson | $\alpha = 0.25$ $\beta = 16.2$ | Haas et al., 1999 |

^a These calibrations are based on available data that have used particular pathogen strains processed in particular ways. Where more than one strain of an organism has been studied in clinical trials, a wide range of infectivities can be discovered. Therefore it must be recognized that these calibrations can carry a substantial degree of uncertainty.

^b For the exponential distribution $N_{50} = 0.693/r$; for the beta-Poisson distribution $N_{50} = \beta * (2^{1/\alpha} - 1)$.

^c Developed for inhalation exposure to adenovirus 4 aerosols.

^d Estimated based on ID₅₀ reported for the TAMU isolate.

^e Represents a meta-analysis of seven outbreaks and adjusting for heterogeneity. Alpha/beta pairs derived via MCMC analyses are available from Dr. Teunis. Use of those pairs is preferred to the use of the values shown in this table

^f Corresponding dose units are grams of feces.

^g Dose-response relation for the conditional probability of illness in infected subjects = $1 - (1 + \eta CV)^{-r}$, where η and r are shown in the Table; CV is the dose (concentration \times volume).

^h An alternate dose-response model is proposed by Brynestad and Braute (2008). That model is not included in Table 4 however, the model is described along with other empirical models in Appendix G.

ⁱ Coleman and Marks (2004) suggest the dose-response models for *Campylobacter* identified in this table do not account for strain variability sufficiently and suggest the need for development of more detailed mechanistic models.

the analysis phase and the risk characterization phase. As indicated in Section 3.3 (Exposure Profile), the iterative nature of risk assessment requires that the host pathogen profile and the exposure profile be critically evaluated by the risk assessors and managers to determine if the problem formulation component of the risk assessment needs to be revisited and refined based on the availability of relevant data presented in these profiles. It should be clear that the quantity

and quality of data that will be available for any particular risk assessment will necessarily vary, nevertheless, the output from the host-pathogen profile serves as subsequent input to the exposure component of the analysis phase and/or the risk characterization phase of the microbial risk assessment.

5. Risk Characterization

As noted throughout the preceding chapters, risk assessment is an iterative process. During risk characterization, the results of this iterative risk assessment process are integrated and documented in a risk characterization summary. Thus, risk characterization is the final step of the MRA process in which all preceding analyses (i.e., infectious disease hazard, exposure, and dose-response assessments) are combined to convey the overall conclusions about potential risk to humans. For these reasons, the risk characterization needs to be complete, informative, and useful for decision-makers.

Risk characterization forms the starting point for formulating risk management considerations and provides a foundation for (regulatory) decision-making. It characterizes both quantitative and qualitative data in technical and non-technical terms, explaining the extent and weight of evidence, results, and major points of interpretation and rationale. It also summarizes the strengths and weaknesses of the evidence, conclusions, uncertainties, variability, potential impact of alternative assumptions, and discusses scenario, model, parameter, and analysis options that may deserve further consideration as the results from the assessment are subsequently used for decision-making purposes.

EPA's Policy Statement on risk characterization (EPA, 2000b) is as follows:

Each risk assessment prepared in support of decision-making at EPA should include a risk characterization that follows the principles and reflects the values outlined in this policy. A risk characterization should be prepared in a manner that is clear, transparent, reasonable and consistent with other risk characterizations of similar scope prepared across programs in the Agency. Further, discussion of risk in all EPA reports, presentations, decision packages, and other documents should be substantively consistent with the risk characterization. The nature of the risk characterization will depend upon the information available, the regulatory application of the risk information, and the resources (including time) available. In all cases, however, the assessment should identify and discuss all the major issues associated with determining the nature and extent of the risk and provide commentary on any constraints limiting fuller exposition.

5.1 Introduction to Risk Characterization

As indicated above, the Agency's Risk Characterization Policy calls for a transparent process and documentation that is clear, consistent, and reasonable. This section provides a summary overview that is intended to provide risk assessors, risk managers, and other decision-makers an introduction to the goals and principles of risk characterization, the essential elements to address in a risk characterization for microbial contaminants, and the various forms risk characterization can take for different purposes. More comprehensive documentation on the topic of risk characterization has been prepared by the Agency and interested readers are referred to EPA (2000b). This MRA Protocol complements and extends that previous work by discussing tools, methods, and issues specific to microbial contaminants in water and water-related media.

5.1.1 Historical Context

The first significant reference to risk characterization is found in the 1983 NRC publication titled *Risk Assessment in the Federal Government: Managing the Process* (commonly referred to as the “Red Book”). In that seminal work, the NRC defined risk characterization as

...the process of estimating the incidence of a health effect under the various conditions of human exposure described in exposure assessment. It is performed by combining the exposure and dose-response assessments. The summary effects of the uncertainties in the preceding steps are described in this step.

Since its publication, the concept of risk characterization evolved within EPA and also more broadly within the U.S. Federal government. For example, in 1984, greater emphasis was placed on making the risk assessment process transparent, on providing a fuller description of the strengths and weaknesses of the assessment, and on providing plausible alternatives within the assessment (Omenn et al., 1997).

Concerns over adequately characterizing risk to maintain the public’s perception of and confidence in EPA’s risk assessments resulted in a 1992 Agency-wide policy for risk characterization, which stated that “...scientific uncertainty is a fact of life (and)...a balanced discussion of reliable conclusions and related uncertainties enhances, rather than detracts, from the overall credibility of each assessment...” (EPA, 1995a).

In 1996, the NRC refined the definition of risk characterization as “...a synthesis and summary of information about a potentially hazardous situation that addresses the needs and interests of decision makers and of interested and affected parties. Risk characterization is a prelude to decision making and depends on an iterative, analytic-deliberative process.” They then proceed to refer to risk characterization as “the process of organizing, evaluating and communicating information about the nature, strength of evidence and the likelihood of adverse health or ecological effects from particular exposures” (EPA, 1995a).

In 1997, the Presidential Commission on Risk Assessment and Risk Management noted the following (Omenn et al., 1997):

Risk characterization is the primary vehicle for communicating health risk assessment findings. Many risk characterizations have relied primarily on mathematical estimates of risk to communicate risk assessment findings, often conveying an unwarranted sense of precision while failing to convey the range of scientific opinion. They are particularly difficult for audiences unfamiliar with risk assessment to comprehend. Effective risk management is impeded without effectively communicating information about who is at risk, how they might be affected, what the severity and reversibility of adverse effects might be, how confident the risk assessors are in their predictions and other qualitative information that is critical to decision-making.

Risk characterization at EPA is considered to be a conscious and deliberate process to bring all important considerations about risk (the likelihood of the risk and also the strengths and limitations of the assessment) and a description of how others have assessed the risk into an integrated picture. As an integrated picture, the risk characterization focuses on how those

components interact (EPA, 2002b). Based on the experiences across the Agency between 1995 and 2000, a single Agency-wide document was determined to be needed. The *Risk Characterization Handbook* (EPA, 2002b) was developed to respond to that need and remains current. However, the *Risk Characterization Handbook* indicates that Agency offices may wish to prepare tailored guidance that meets their individual needs to supplement and remain consistent with the information in the Handbook. This MRA Protocol fills one such need as the field of MRA has evolved rapidly over the past several decades.

5.1.2 Elements of Risk Characterization

Risk characterization consists of two major steps—risk estimation and risk description. Risk estimation is the compilation of the types and magnitude of effects anticipated from exposure to the microbe or medium and can be qualitative or quantitative depending on the data and methods used.

Logistically, the risk estimation is derived from the output from the analysis phase for exposure characterization and human health characterization. Specifically, the results from the characterization of exposure can be expressed as the number of organisms to which an individual is exposed in a defined amount of time and/or for a certain consumption rate. The results from characterization of human health effects can be expressed as the probability of individual illness after a certain number of organisms are consumed. The risk estimation can be expressed as an individual risk estimate (e.g., 1 per 1000 probability of illness) or as a population level risk estimate (100 illnesses per year in a region with a population of 100,000 individuals). As described in further detail below, the risk estimation can also be modeled to consider time-dependent elements such as secondary (person-to-person) transmission, host immunity, and multiple routes of exposure (ILSI, 2000). Aspects of and considerations for risk estimation are discussed below in Sections 5.1.3, 5.2, 5.3, and 5.4.

The second component of risk characterization, risk description, involves summarizing the event of interest according to its nature, severity, and consequences. The risk description also includes a discussion and quantifications (to the extent possible) of (1) the uncertainties associated with the problem formulation, analysis, and key components within the risk characterization; (2) the variability associated with key inputs to the model(s); (3) the confidence in the resulting risk estimates through a weight of evidence discussion; (4) the limitations of the analysis; and (5) the plausibility of the results. Important aspects for the risk description are described in Section 5.5

5.1.3 Parsimony

The first mathematical models to analyze the spread and control of infectious diseases were developed in the early 20th Century in attempts to understand measles (Hamer, 1906) and malaria (Ross, 1911). Quantitative methods to characterize human health risks specifically associated with exposure to pathogenic microorganisms were first published in the 1970s (Dudley et al., 1976; Fuhs, 1975). This field grew exponentially in the middle of the 20th Century. A tremendous variety of models have now been formulated, mathematically analyzed, and applied to infectious diseases (Hethcote, 2000). Mathematical models of disease transmission have become important tools that have led to understanding the transmission characteristics of

infectious diseases in communities and better approaches to decreasing the transmission of these diseases (Hethcote, 2000; King et al., 2008; Riley et al., 2003).

Since the 1970s many MRAs have used the 1983 NRC risk assessment framework for chemicals as a basis for waterborne (e.g., Crabtree et al., 1997; Gerba et al., 1996b; Haas, 1983; Mena et al., 2003; Regli et al., 1991; Rose et al., 1991; Teunis et al., 1997) and foodborne pathogen assessments (Buchanan et al., 1998, 2000; Farber et al., 1996). Consistent with the chemical risk framework, most of these assessments have assumed that the number of individuals that are susceptible to infection is not time varying (static) and, thus risk is characterized at an individual level (Eisenberg et al., 2002). Static models have also been used by the Agency in the development of drinking water regulations (EPA, 2002e, 2006).

As the field of MRA developed, the advantages of modeling infectious disease processes such as person-to-person transmission of infection and immunity became increasingly apparent (Eisenberg et al., 1996, 1998). Addressing these issues requires dynamic methods where the number of individuals that are assumed to be susceptible to infection is time-varying and risk is manifest at the population level (Anderson and May, 1991; Hethcote, 1976, 2000). EPA recognized these needs and initiated the development of an MRA framework that explicitly acknowledges that aspects that are unique to the transmission of infectious diseases could be important for risk assessment for these organisms (ILSI, 2000).

From a modeling perspective, biological “realism” is often counter-balanced by analytical complexity. The increase in the complexity of a model structure increases variability due to the uncertainties associated with model specification and/or increases the computational demands (EPA, 2004c). On the other hand, a simpler model form involves implicit or explicit assumptions that may or may not be realistic or appropriate for a particular situation. For the purposes of this MRA Protocol, the concept of parsimony is encouraged; that is, models should be as simple as possible, but no simpler. Within this context, more complex models should be considered or used under conditions in which the added complexity may provide sufficient additional insight that the additional complexity is warranted (King et al., 2008; Soller and Eisenberg, 2008).

Surprisingly little research has been conducted to date that evaluates the applicability of different types of models under different pathogen-exposure combinations. One published study in this arena indicates that there may be conditions where the results from two of the more common MRA modeling approaches yield similar results (Soller and Eisenberg, 2008). Within the context of parsimony, these conditions are of particular interest. In selecting a MRA model, caution must be taken to ensure that any simplifying assumptions that are employed are in fact appropriate from an epidemiological perspective. Within that context, and to the extent possible, MRAs should use epidemiological data as fundamental components of the assessment and should demand higher quality input data and fewer simplifying assumptions when seeking increased risk assessment accuracy and precision.

5.2 Representative Model Forms for MRA Risk Estimation

A variety of model forms can be employed for the assessment of infectious disease transmission

and the potential impact or benefit of intervention efforts/management actions. Particular characteristics of each model form allow for the capture of different aspects of the disease transmission system (EPA, 2004c). In the following sections, several of the most commonly employed models are summarized and reviewed. Exclusion from the following discussion should not preclude use of a particular model form; however, justification for use of a particular model form should be included in the risk description (see Section 5.1.2). An overview of two commonly employed classes of MRA models is provided in Table 5, while an overview of model types developed previously by EPA is provided in Figure 9 (EPA, 2004c).

5.2.1 Static Models

Some infectious diseases are not readily transmitted from person-to-person but are acquired, to the best of current knowledge, only by consumption of, or contact with, contaminated environmental materials (e.g., MAC infection from drinking water). In other cases, although an agent may have the potential to be transmissible, the particular situation is such that the person-to-person component is unknown or thought to be negligible.

Understanding the pattern of human infections from such pathogens or exposure scenarios may be best achieved through the use of static models (parallel to those used for toxicological risk assessments). The chemical risk assessment-based models are used to estimate risk at an individual level and typically focus on estimating the probability of infection or disease to an individual as a result of a single exposure event. With respect to microbial contaminants in

Table 5. Overview and Comparison of Static and Dynamic Risk Assessment Models

| Static Risk Assessment Model | Dynamic Risk Assessment Model |
|--|---|
| Number of susceptible individuals is time invariant | Number of susceptible individuals varies over time |
| Direct exposure (environment-to-person) | Direct and indirect exposure (environment-to-person person-to-person, and person-to-environment-to-person) |
| Individual-based perspective | Population-based perspective |
| Typically assumes that the potential for secondary transmission of infection or disease is negligible or scales linearly with the number of infections | Typically account for the potential for secondary or person-to-person transmission of infection or disease |
| Typically assumes that immunity to infection from microbial agents is negligible | Exposed individuals may not be susceptible to infection or disease because they may be infected already or may be immune from infection due to prior exposure |
| Dose-response function is the critical health component | The dose-response function is important; however, factors specific to the transmission of infectious diseases may also be important |

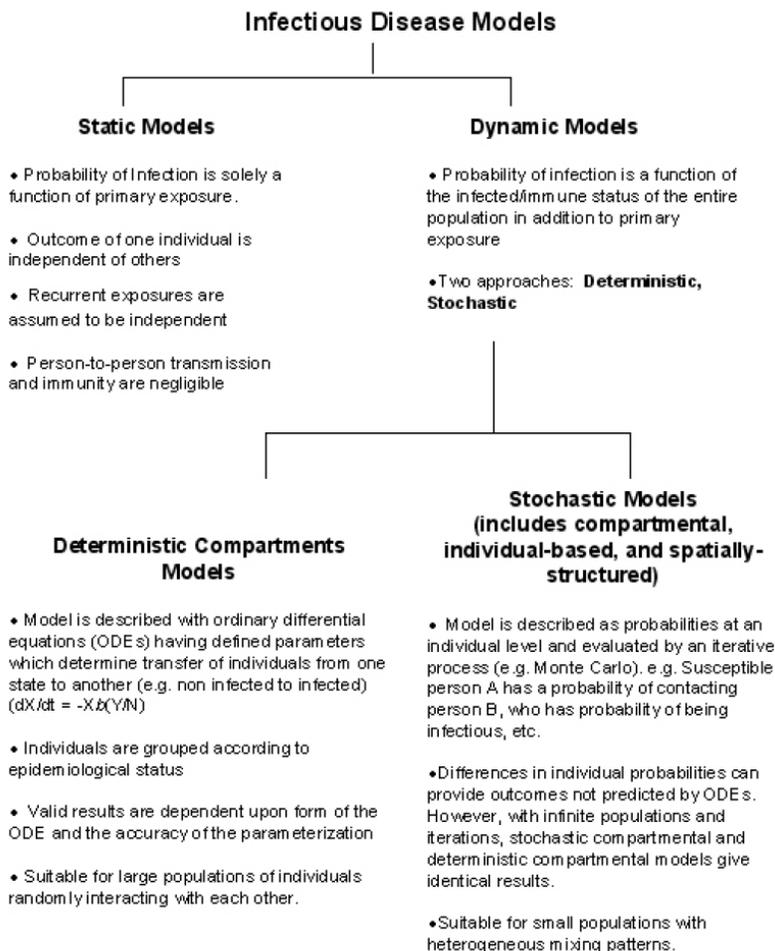


Figure 9. Overview of Infectious Disease Model Types
(Source: EPA, 2004c)

water, a fundamental simplifying assumption of static model-based analysis is that exposure events and infection/disease are independent; that is, the outcome from one exposure event does not affect a subsequent exposure, and one individual’s outcome has no impact on any other individual’s outcome. Thus, secondary transmission and immunity are most often assumed to be negligible or are of similar magnitude and effectively cancel each other out. (It is generally assumed that secondary transmission would increase the level of infection/disease in a community relative to a specific exposure to pathogens, and immunity would decrease the level of infection/disease in a community relative to a specific exposure to pathogens.)

A static model would be appropriate in those cases where the central question is concerned with the probability of infection or illness relative to the dose of pathogens acquired from a single exposure. Such models can handle complex details about the course of events that lead to exposure and infection and can be analyzed by well established statistical techniques that require fewer assumptions than do dynamic models (discussed below). Static models are useful for analyzing situations where the effect of an intervention directed to individuals (e.g., point-of-use remediation) is more important than the effect on transmission throughout the population; they

are not appropriate for measuring indirect effects at the population level (e.g., the effect of water treatment interventions on risk due to secondary transmission).

A review of the scientific literature indicates that static models (NRC, 1983) have been commonly used as a generic framework for conducting MRAs related to water- and foodborne pathogens (Buchanan et al., 1998, 2000; Crabtree et al., 1997; Farber et al., 1996; Gerba et al., 1996b; Haas, 1983; Mena et al., 2003; Regli et al., 1991; Rose et al., 1991; Sanaa et al., 2000; Teunis et al., 1997; Voysey and Brown, 2000). Moreover, most drinking water regulations in the United States have been primarily based on static models. In most static models, it is assumed that the population may be categorized into two epidemiological states—a susceptible state and an infected or diseased state. In these models susceptible individuals are exposed to the pathogen of interest and move into the infected/diseased state with a probability that is governed by the dose of pathogen to which they are exposed and the infectivity (dose-response relationship) of the pathogen.

A representative conceptual model for a static MRA model is presented in Figure 10. As can be seen, individuals who are exposed to pathogens from a specific source, move from a susceptible state into an infected or diseased state with some probability that is governed by their exposure and the dose-response relationship for that pathogen. Also note that previous exposures to the pathogen, interactions with other (potentially infected) individuals, other routes of exposure, and immune status are not included in this type of model.

5.2.2 Dynamic Models

Risk managers and regulators are often concerned with risk on a societal or population scale. Thus, individual risks need to be translated to the level of the exposed population or some other relevant part of that population. When an infectious agent that occurs in water is also contagious, its impact on a population can be significantly influenced by the interactions between contagious and susceptible individuals. To assess the full impact of human exposure to pathogens, infectious disease risk assessors need to address risk at the population level in addition to individual risk at the dose-response level. For a thorough evaluation of risks that are manifest at the population level, MRA methods must explore the relative importance of secondary transmission and immunity, and thus capture and integrate the dynamic interplay of hosts, agents, and environments.

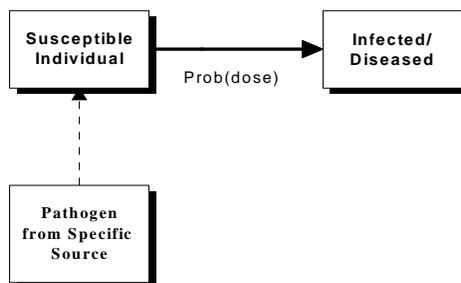


Figure 10. Static Risk Assessment Conceptual Model

Dynamic MRA models take two main forms—deterministic or stochastic. “Deterministic” means that the model output is strictly determined by the starting conditions and the values of the parameters in the equations that define the system. In stochastic models, events are treated as stochastic (random) events rather than deterministic ones. Dynamic MRA methods have been used for numerous specific case studies in the United States (Eisenberg et al., 1996, 1998; Koopman et al., 2002; Soller et al., 1999, 2003, 2006) and recently to support regulatory decisions by EPA (EPA, 2006b). However, stochastic MRA models are still research tools that continue to undergo development.

Deterministic Dynamic MRA Models

Deterministic dynamic MRA models are suitable for large populations of individuals randomly interacting with one another (see Text Box 11). In this form, the population is divided into one of the following different epidemiological states: (1) susceptible, (2) diseased (infectious and symptomatic), (3) carrier (infected but asymptomatic), and (4) immune (partial or complete). Only a portion of the population is in a susceptible state at any point in time, and only those individuals in a susceptible state can become infected through exposure to pathogens. The dynamic aspect of the model means that members of the study population move between epidemiological states at different rates, and thus, the number of individuals in each state changes over time.

Variables in the model track the number of individuals that are in each of the epidemiological states at any given point in time (thus, these variables are called state variables). The sum of the number of individuals in each of the epidemiological states equals the total population. A representative conceptual model for this type of MRA model is presented in Figure 11 while the corresponding parameters for this model are presented in Table 6.

Text Box 11: Population Mixing Patterns

(Source: Adapted from EPA, 2004c)

When modeling infection transmission, it may be important to account for the different ways in which individuals may come into contact with one another. In these cases, it may be important to consider both how individuals make contact and with whom that contact is made. Basic population mixing patterns include the following:

- Random homogeneous mixing—the simplest pattern, occurs when every person has an equal chance of making contact with every other person and consequently an equal chance of exposure to infection. This pattern has been referred to as global mixing
- Heterogeneous mixing—can occur from many different patterns of interactions, in which a susceptible individual is more or less likely to contact an infective individual based on probabilistically determined patterns of interactions and contact likelihoods for specific subgroups at risk; some heterogeneous patterns include:
 - Local mixing—is exhibited when the assumption modeled is that there is a contact distribution centered on an infective individual and contact is made only with nearby neighbors, such as family members, age groups, and school classes.
 - A household model captures one form of local mixing. In this model the population is partitioned into households and local contacts are chosen randomly from within an infectious household.
 - Disseminating mixing—occurs when contacts are made between communities via a common mixing site in a manner that affects everyone.

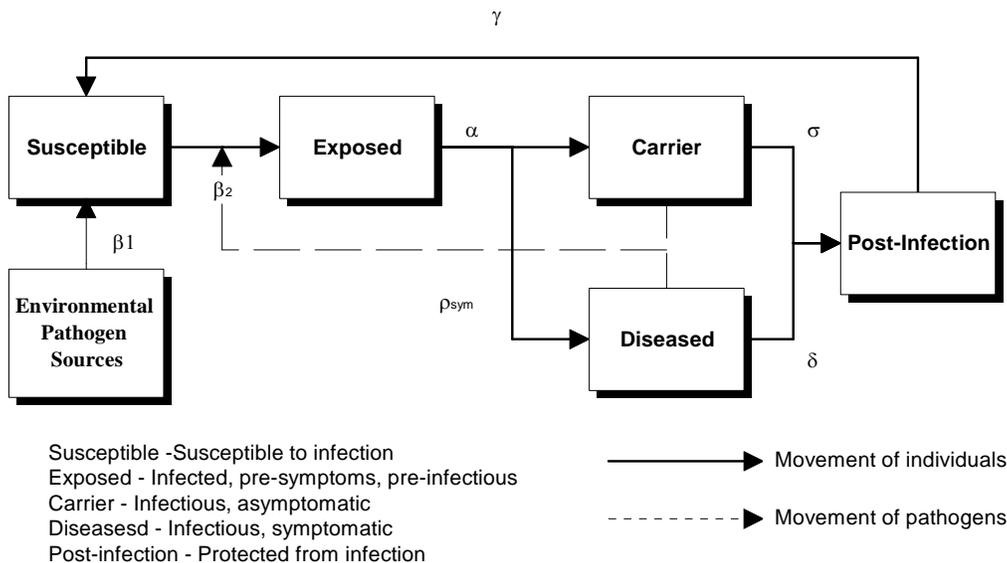


Figure 11. Dynamic Risk Assessment Conceptual Model
 (Source: Soller, 2008; Soller and Eisenberg, 2008)

Table 6. Summary of Model Parameters for Deterministic Dynamic MRA Model
 (Source: Soller, 2008; Soller and Eisenberg, 2008)

| Parameter | Model Parameter |
|--------------|---|
| ζ | Duration of incubation |
| δ | Duration of symptomatic infection |
| σ | Duration of asymptomatic infection |
| γ | Duration of protection from infection |
| ρ_{sym} | Probability of symptomatic response |
| β_1 | Rate that individuals move out of State S due to exposure to pathogens from environmental (primary) source |
| β_2 | Rate that individuals move out of State S due to exposure to pathogens from person-to-person (secondary) transmission |

Deterministic dynamic MRA models are expressed mathematically as a set of differential equations. These equations describe the rate of change in the number (or density) of individuals in a particular state (or compartment) over time and have defined parameters and starting conditions. For example, the equations used to express the model shown in Figure 11 are presented in Figure 12. Rate parameters (i.e., the Greek letters in Figure 11) determine the population’s movement from one state to another. Factors affecting the population dynamics include the level and frequency of exposure, the ability of individuals in infectious states to infect susceptible individuals, and the temporal processes of the disease (e.g., incubation period, duration of disease, duration of protective immunity, etc.). The rate parameters may be determined through literature review or through site-specific data, if available and appropriate.

$$\begin{aligned} \frac{d}{dt} S(t) &= -\beta \cdot S(t) - \beta_{pp} \cdot S(t) \cdot (C(t) + D(t)) + \gamma \cdot P(t) \\ \frac{d}{dt} E(t) &= \beta \cdot S(t) + \beta_{pp} \cdot S(t) \cdot (C(t) + D(t)) - \zeta \cdot E(t) \\ \frac{d}{dt} C(t) &= \zeta \cdot (1 - psym) \cdot E(t) - \sigma \cdot C(t) \\ \frac{d}{dt} D(t) &= \zeta \cdot psym \cdot E(t) - \delta \cdot D(t) \\ \frac{d}{dt} P(t) &= \delta \cdot D(t) + \sigma \cdot C(t) - \gamma \cdot P(t) \end{aligned}$$

Figure 12. Differential Equations Used to Express Dynamic Model

(Source: Soller, 2008; Soller and Eisenberg, 2008)

Deterministic dynamic MRA models have a number of limitations. If they are used to model relatively small populations, the assumption of homogeneous mixing of the individuals in the population can lead to mis-estimation of disease. These models also require appropriate parameter values for transmission rates (β_2 in Table 6) and such information can be quite difficult to determine accurately. Lack of knowledge and data, as well as inherent biological variability, suggest a need for uncertainty and sensitivity analyses of parameter values. Furthermore, random events such as local introduction or local die-out of a disease in a neighborhood of a heterogeneously mixing population are difficult to incorporate into these models (EPA, 2004c).

Finally, comparison of the conceptual models for the static and deterministic dynamic models indicates that under a specific set of assumptions the two models are essentially equivalent (Soller et al., 2004). Those conditions are ones in which a static model would yield similar results to a deterministic dynamic models and are as follows:

- the background concentration of the pathogen (or equivalently the endemic level of infection/disease) in the population is zero or unimportant;
- the duration of infection and disease approaches zero; and
- infection and/or disease do not confer immunity or the duration of immunity approaches zero.

5.2.3 MRA Model Forms Under Development

Stochastic Dynamic MRA Models

In a stochastic form, dynamic models incorporate probabilities at an individual level and are evaluated by an iterative process (e.g., susceptible person A has a probability of contacting person B, who has a probability of being infectious, etc.). This type of model also uses states (or compartments) for classifying the epidemiological status of the population and subpopulations (e.g., human immunodeficiency virus [HIV]-positive individuals, individuals greater or less than 5 years of age) under study, but differs from the deterministic dynamic MRA models in that the compartments contain discrete individuals rather than the numbers or densities of persons that are represented by the compartments in deterministic dynamic MRA models.

In stochastic dynamic MRA models, events are treated as random (stochastic) events rather than deterministic ones. These models employ distributions of outcomes rather than the average outcomes as do the deterministic models; a stochastic model will produce different results each time it is run—even with the same starting conditions and parameters due to the effects of chance. Stochastic forms are suitable for small populations and heterogeneous mixing patterns where stochastic events can have a major impact. In a small population, chance events, such as an infectious person contacting only immune persons during the infectious period of illness, may have a substantial impact on the transmission dynamics of the disease (EPA, 2004c).

These types of models have been used to investigate the stochastic effects of disease transmission and localized exposure (EPA, 2004c). For example, King et al. (2008) used a nonlinear stochastic model coupled with a new likelihood maximization procedure for model parameter values to explain the dynamics of cholera infection in Bengal, the pathogen's endemic home.

Individual-Based (or Individual Event History) Models

Individual-based models (also known as microsimulation models) are a subset of stochastic dynamic MRA models that count individuals and consider the history experienced by each person. In general, stochastic dynamic models require that all combinations of individual traits be defined in advance for implementation in the model structure, whereas individual-based models allow for these combinations to evolve in the model execution. Unlike deterministic compartmental models (as described above), in an individual-based model, transmission occurs between specific individuals. Individual-based models are useful for populations with large amounts of important heterogeneity and where life history is important (i.e., when the studied process has memory). One principal limitation of these models is that they require considerable computational power.

Spatially-Structured Models

For some diseases, the spread of disease across a geographical area is important because of the impact the spatial situation has on contact and mixing patterns. A spatially limited mixing structure for a dynamic network model could describe an exposure scenario in which some of the

contacts that an individual makes are at a neighborhood level while other contacts that individual makes are common across a geographic region (e.g., all the neighborhoods are served by a common drinking water source) (Koopman et al., 2002; Soorapanth et al., 2001). Such a spatially-defined setting changes the spread of disease in comparison to what would be predicted by a compartmental model. Simple models of this type locate each individual at a point in a lattice and each susceptible individual's risk of infection depends on the infection status of his nearest neighbors; these are discrete dynamic systems whose behavior is completely specified in terms of local contacts.

5.3 Data Representation in MRA Risk Estimation Models

In assessing risk associated with infectious disease hazard exposures, it is necessary to estimate a number of parameters in the risk models. Depending on the data quality, different representations of these data (as discussed below) may be appropriate. For some chemical risk assessments, EPA has made a policy decision that conservative estimates (i.e., high-end estimates assumed to be health protective) of some exposure factors should be used to assure the desired level of health protection for sensitive segments of the exposed population (EPA, 2000c). The Agency has not developed a comprehensive policy with regard to the conservativeness of parameter estimates in MRA. In fact, the use of multiple layers of conservative estimates for microbial contaminants has been shown to result in risk estimates that are not credible and that are overly protective (EPA, 1995b). Thus, the selection of values used in the risk characterization and the respective data representation should be well documented in the risk description. The following is an overview of the various ways that data can be represented in an MRA.

Point Estimates

A "point estimate" is a single-valued estimate of a parameter used in risk assessment. Using point estimates for all the parameters in a risk equation results in a single value (point estimate) of risk that provides no information concerning the potential sources of variability or uncertainty or the magnitude of that uncertainty associated with the risk estimate. Lack of information regarding potential variability and uncertainty in the quantity being estimated is a fundamental weakness of the point estimate approach. The strength of using point estimates is the relative ease of use and simplified risk assessment output. For example, all of the equations for estimating risk and deriving criteria values for EPA's 1986 Bacterial AWQC use point estimates of input parameters³¹ (EPA, 1986a). In some cases, the point estimates themselves may be selected taking the potential uncertainties in the parameter values into consideration. For example, EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (EPA, 2000c) recommends that 90th percentile estimates derived from national studies of drinking water and fish consumption by adults be used in estimating criteria values for chemicals.

Confidence limits provide an indication of the degree of uncertainty associated with a statistic (Snedecor and Cochran, 1989). Although they are usually derived for estimates of the arithmetic

³¹ The criteria themselves were based on point estimates; however, the numerical guidance for interpreting single analyses were based on lognormal confidence limits.

mean, they can also be estimated for other statistics (e.g., median and percentiles). The narrower the interval, the more precisely the statistic has been estimated. The magnitude of uncertainty is expressed in the form of upper and lower confidence limits (collectively known as the confidence interval); confidence limits always have an associated confidence level (e.g., 90%, 95%, etc.). The confidence level reflects the estimated probability that the numeric statistic estimated, based on a sample of a given population size, will fall within the specified confidence interval. Confidence limits typically assume that the underlying distribution in the study population is “normal” (Gaussian), but alternative assumptions can also be used. Confidence limits can also be derived that make no assumptions (nonparametric) about distribution shape. An example of a confidence limit used in risk characterization can be found in EPA’s *Risk Assessment Guidance for Superfund* (EPA, 1989), where the 95% upper confidence limit on the arithmetic mean soil concentration is recommended as the appropriate point estimate for a screening level risk assessment at Superfund sites.

Statistical Distributions

If adequate data are available, it may be possible to accurately characterize the statistical distribution of a parameter used in risk assessment. That is, there may be enough data to select the form of the distribution and to accurately estimate its parameters (e.g., mean, standard deviation, percentile values for a normal or lognormal distribution). Where such data are available (examples include national surveys of water intake and body weight), individual summary statistics can be estimated very accurately (confidence limits are narrow).

Bayesian Methods

Bayesian methods were introduced in Section 4.3.3 with respect to their use in dose-response modeling and are discussed more fully in [Appendix G](#). In this section, additional uses of Bayesian methods within QMRA are described, with emphasis on applications of Bayesian methods to quantify uncertainty or applications that leverage data to reduce uncertainty in exposure assessment and dose-response modeling. The studies described below are presented, not as a comprehensive listing of application of Bayesian techniques in quantifying uncertainty in QMRA, but as illustration of the use of these techniques.

The same methods that were described for their use in dose-response modeling can also be used to characterize the uncertainty in model parameters through the generation of “uncertainty samples.” These uncertainty samples are particularly useful in MRA because they characterize fully the uncertainty for a specific model parameter, given the available data. For example, Messner et al. (2001) combined three *Cryptosporidium* isolates that were considered representative of a larger population of human-infecting strains and determined that the risks of infection produced from single oocyst doses for a mixture of the three isolates and for an oocyst selected at random from the larger population of strains were 0.018 and 0.028, respectively. A related uncertainty analyses was conducted for the MRA that was conducted for the economic analysis of the LT2 drinking water regulation (EPA, 2006a).

Hierarchical Bayesian modeling is often used in MRAs to combine results from different studies or isolates in a meta-analysis. For each study or isolate, the parameters can be randomly selected

from a distribution that depends upon several additional parameters, called hyperparameters. For example, the single oocyst infection probability for each *Cryptosporidium* isolate can be modeled as being randomly drawn from a normal distribution with hyperparameters μ and σ that represent the variability of the isolate infectivity across the isolate population (Messner et al., 2001). As noted previously, MCMC methods can accommodate hierarchical Bayesian models.

Gronewold et al. (2009) demonstrated that Bayesian techniques can be used for quantifying and analyzing uncertainty in exposure model fate and transport parameters. In that study, Bayesian methods for addressing uncertainty and developing models were compared with regression techniques in which a model was assumed and uncertainty was assumed related to confidence in the estimates of model parameters. In comparing approaches for estimating decay rate parameters from microbial survival experiments, Gronewold and colleagues found that Bayesian techniques, because they rely on fewer assumptions about parameter variability than alternative techniques, provided higher estimates of variability in the parameters and likely reflect actual conditions more accurately. Bayesian techniques also allowed these researchers to assess the forms of models proposed for microbial inactivation and to assess alternative models of the process. The work reported in Gronewold et al. (2009) is an extension of prior studies by the authors (Gronewold et al., 2008) in which uncertainty in different enumeration processes was quantified and related to assessment of water quality.

Crépet et al. (2009) and Pouillot et al. (2003) used Bayesian inference to estimate growth model parameters and estimates in the variability for *Listeria monocytogenes* growth on or in different foods (lettuce and milk). Crépet et al. found that, although their model yielded growth parameter mean and standard deviation estimates similar to those generated using a two-stage frequentist estimation approach in which stages of the growth and measurement process are analyzed separately, the benefit of the Bayesian model was its flexibility and ability to accommodate data from diverse sources, as well as the ability to include information about the temperature dependence of the growth rate into their model. In addition to the benefits Crépet ascribe to use of Bayesian techniques, Pouillt et al. (2003) note that Bayesian methods allow inferences on hyperparameters to be made more easily than frequentist approaches and that validated computer programs for performing Bayesian analysis are generally available.

Clough et al. (2003) used Bayesian techniques for estimating parameters in distributions of animal infection prevalence based on fecal pat sampling. For this application, Bayesian techniques were considered superior to traditional estimation approaches because of their ability to account for uncertainty in microbial detection methods and in uneven distribution in fecal production among animals within a herd. The authors assessed that the Bayesian inference was apt for this application, based on the relative insensitivity of the posterior distribution to the choice of prior distribution and based on the consistency of observed predictions of the technique with known trends. Results of the analyses included a methodology for developing improved sampling schemes for fecal pat sampling and herd health assessment. In a subsequent study, Ranta et al. (2005) demonstrated the use of Bayesian inference to develop estimates of *Salmonella* infection at the regional level, herd level, and individual animal level (at slaughter). Similar to Clough et al. (2003), Ranta et al. (2005) noted that Bayesian techniques were particularly useful for this application because the infection process is complex and the Bayesian techniques permitted use of information and knowledge not used in frequentist approaches.

Englehardt and Swartout (2004) derived a hierarchical predictive population dose-response Bayesian assessment for *C. parvum* for the infection endpoint. In that study, available data on the infectivity of three isolates of *C. parvum* were adjusted for sensitive and antibody-positive subpopulations not proportionately represented in the data. The results from this study indicate that the predicted population-level infectivity of *Cryptosporidium* based on a predictive Bayesian dose-response relation adjusted for sensitive and antibody-positive subpopulations, lies generally between the infectivity of the UCP and Iowa isolates. Further, Bayesian techniques allowed incorporation of relevant population information (prevalence of sensitive and antibody positive subpopulations) into estimates, whereas frequentist models that can leverage this type of information have yet to be developed.

In a study related to both exposure assessment and dose-response modeling, Ramachandran (2001) conducted a retrospective exposure assessment to estimate worker exposures to inhalable nickel aerosols during occupational exposure at nickel smelters. Similar techniques could potentially be employed in QMRA for developing dose-response models in the absence of quantal dose-response data (e.g., using outbreak data) or in developing parameter estimates for use in exposure assessment. Ramachandran used a combination of deterministic modeling and expert judgment in formulation of prior distributions and developed posterior distributions using available (historical) measurements. Prior distributions were generated via probabilistic simulations in which the deterministic model was run with model parameters drawn from probability distributions assembled based on expert judgment and available data. Use of Bayesian techniques allowed for uncertainties of historical measurements to be accounted for explicitly, and the use expert judgment to help overcome limitations of a sparse data set.

EPA anticipates that hierarchical modeling will continue to be important in the future of microbial risk assessment. Roles that Bayesian techniques may be expected to play, as illustrated with the examples above, include development of dose-response models in the absence of human dose-response data, parameter estimation for sparse data sets or for data sets exhibiting wide variability, and/or assessment of alternative models, particularly in exposure assessment.

Probabilistic Simulations

Distributional data and/or Bayesian-based uncertainty samples can be used in MRAs by performing probabilistic simulations or related methods. In these types of analyses, risk calculations (each of which yields a point estimate) are repeated many times (typically thousands of times) using random or structured “draws” of values from the distributions of each parameter value. The resulting distribution of risk provides information about the expected precision of the estimate, given the distributions of and/or uncertainty associated with the input parameters. The contributions of variability in individual parameters can also be estimated and the correlations among parameters can be accommodated within a Monte Carlo framework.

EPA has developed guidelines for when probabilistic methods can and should be used in health risk assessments (EPA, 1997b). The most common obstacle to the use of probabilistic modeling (now that computational capacity is no longer a major issue) is the lack of data to adequately characterize the variability and/or uncertainty in key input parameters. One approach that has

been used at EPA is a “tiered approach” to risk assessment, whereby the first step is a set of screening calculations to determine if the risks being estimated fall within the range of concern under a credible set of assumptions. If the results of the screening level analysis warrant further evaluation, sensitivity analyses can be used to further characterize the likely range of risks and to guide data gathering efforts for key parameters. If sufficient data are available, and if more detailed information is needed or desired regarding the decision being evaluated (e.g., setting a health-based criterion), then Monte Carlo modeling may be useful as a subsequent tier.

The decision whether to use probabilistic methods can be technically complex; thus, expert statistical advice should be sought on such decisions. When planning such assessments, it is important to ensure that the approach taken to characterize uncertainty is consistent across the models used in all stages of the risk assessment. A recent example of such an analysis can be found in EPA’s risk assessment in support of the LT2, which addresses *Cryptosporidium* contamination in sources of drinking water (EPA, 2006a).

5.4 Risk Estimation Sensitivity and Uncertainty Analysis

Uncertainty analysis “is the computation of the total uncertainty induced in the output by quantified uncertainties in the inputs and models...” (Morgan and Henrion, 1990). It is a key concern for risk managers because uncertainty analysis provides information about the overall reliability of the risk estimates. Measures of model “uncertainty” communicate to risk managers the risk assessor’s best judgment as to the overall quality of the numerical risk estimates generated by the MRA. Confidence intervals, “credible ranges” developed through Bayesian analyses, and other measures of dispersion in risk estimates, must be presented clearly, and their meaning communicated clearly. Similarly, clear graphical or tabular presentations are very useful. To the extent that intermediate calculations add value and understanding to the results, they can also be included. Key assumptions related to model selection, input data, and parameters should be provided and discussed, as well as their implications for the model results and uncertainty. Any conservative assumptions that are built into the model should be explained and the impact of using less conservative assumptions should be discussed.

In many risk assessments, assumptions and rough estimates for input values and/or uniform and triangular distributions are used to account for uncertainties in input values that cannot be easily quantified. Uncertainty can also stem from the selection of the model form that is used for the risk assessment. One method that has been used to evaluate this source of uncertainty is model averaging (EPA, 2006a).

It is also important to carefully evaluate the impact of known sources of variability in model outputs. This is generally done through use of one or more forms of sensitivity analysis. Sensitivity analysis “is the computation of the effect of changes in input values or assumptions (including boundaries and model functional form) on the outputs” (Morgan and Henrion, 1990). Sensitivity analyses techniques range from simply conducting a small number of additional model runs with different parameter values to performing a fully probabilistic evaluation of the effects of variations in parameter values on model outputs. The specific approach that is taken will depend on the nature of the data and models supporting a given assessment. The USDA (Frey et al., 2004) identified several sensitivity analytical techniques useful for MRA (Table 7).

Table 7. Approaches Recommended by USDA for Sensitivity Analysis in Microbial Risk Assessment (Source: Adapted from Frey et al., 2004)

| Method | Procedure |
|---|---|
| Mathematical Methods | |
| Nominal range sensitivity analysis (NRSA) | Vary values of individual input variables across entire range on “nominal” (plausible) values, evaluate effects on model output |
| Differential sensitivity analysis (DSA) | Vary values of individual input variables within small range near central tendency values, estimate importance from “local” change in model output |
| Statistical Methods | |
| ANOVA (analysis of variance) | Stratify values of individual variables into ranges, conduct analysis of variance of model outputs using the strata as “treatments”; use F-statistic values to compare and rank importance of variables |
| Sample and rank correlation/regression | Evaluate correlations/regression results between individual variables and model output; variables with highest correlations/most significant regression slopes are most important |
| CART (classification and regression tree) | Identify variables, combinations of variables, and cutoff values that best divide the data into two groups; repeat until no more statistically significant differences between groups can be detected. “Prune” less significant branches of the “tree” to preserve the most significant differences (variables, cutoff values that best predict model outputs). |
| Graphical Methods | |
| Scatter plot | Plot (transformed) individual variable values against (transformed) model outputs; examine plot for relationships |
| Conditional plot | Plot model outputs versus ranges of values for individual variables, holding other variables constant at representative values; illustrates the effect of the variable on model output, given realistic values of other inputs |

Although the USDA study focused on assessing microbial risks associated with food processing, the general approaches summarized in Table 7 are also applicable to MRAs for other media, including water and water-related media. The methods range from simple and intuitive (varying input values across their observed ranges, scatter plots) to more complex statistical procedures (e.g., classification and regression tree [CART]). For any given risk assessment, it is likely that more than one of these methods will be useful for sensitivity analysis.

Although sensitivity analyses are useful for evaluating the effects of the variability in single parameters on risk estimates, when multiple parameter values vary, the results of sensitivity analyses must be interpreted cautiously (EPA, 1997a). If the variations in parameter values are independent of one another, it is easy to overestimate the impact of varying more than one value because using upper or lower percentile values for more than one variable can yield point estimates of risk that are overly conservative or insufficiently protective. If the variability in risk parameters is correlated, the impact of their variations may not be easy to estimate using sensitivity analysis. In such cases, a more detailed and comprehensive analysis may be required,

usually employing probabilistic approaches such as Monte Carlo or related simulation techniques. Where the variability in model parameters can be partitioned into components mainly reflecting variability and uncertainty, “two-dimensional” Monte Carlo analysis can be employed to estimate the relative importance of these two components. (Refer to EPA [2006] for an excellent example of a two-dimensional Monte Carlo analysis.) Monte Carlo analysis and the usual “diagnostics” that it generates can also be used both to estimate the overall precision in model outputs and to identify those input parameters that contribute the most to the overall variability in the risk estimates (FAO/WHO, 2003; Frey et al., 2004).

Although the USDA (Frey et al., 2004) method for grouping sensitivity analyses into mathematical, statistical, and graphical methods is useful, it is important to note that other authors and reports have provided alternative descriptive groups. For example, the EPA *Exposures Factors Handbook* (EPA, 1997a) provides several approaches to quantitative uncertainty and sensitivity analysis (see Table 8). In addition, Morgan and Henrion (1990) discuss in detail the following four techniques for sensitivity and uncertainty analysis, including:

- **deterministic**, one-at-a-time analysis of each factor holding all others constant at nominal values;
- **deterministic joint analysis**, changing the value of more than one factor at a time;
- **parametric analysis**, moving one or a few inputs across reasonably selected ranges such as from low to high values in order to examine the shape of the response; and
- **probabilistic analysis**, using correlation, rank correlation, regression, or other means to examine how much of the uncertainty in conclusions is attributable to which inputs.

Table 8. Approaches to Sensitivity and Uncertainty Analysis Recommended in EPA’s Exposure Factors Handbook (Source: EPA, 1997a)

| Approach | Description | Example |
|------------------------------------|---|--|
| Sensitivity analysis | Changing one input variable at a time while leaving others constant to examine affect on output | Fix each input at lower (then upper) bound while holding others at nominal values (e.g., medians) |
| Analytical uncertainty propagation | Examining how uncertainty in individual parameters affects the overall uncertainty of the exposure assessment | Analytically or numerically obtain a partial derivative of the exposure equation with respect to each input parameter |
| Probabilistic uncertainty analysis | Varying each of the input variables over various values of their respective probability distributions | Assign probability density function to each parameter; randomly sample values from each distribution and insert them in the exposure equation (Monte Carlo simulation) |
| Classical statistical methods | Estimating the population exposure distribution directly, based on measured values from a representative sample | Compute confidence interval estimates for various percentiles of the exposure distribution |

EPA's *Guiding Principles for Monte Carlo Analysis* (EPA, 1997b) provides guidance on selection and development of the conceptual and mathematical models, selecting and evaluating input data and distributions, evaluating variability and uncertainty, and presenting the results of Monte Carlo analysis. In addition to a policy statement for the use of probabilistic analysis in risk assessment at EPA, eight "conditions for acceptance," which are also reflected throughout this MRA Protocol, are outlined and reproduced below:

1. The purpose and scope of the assessment should be clearly articulated in a problem formulation section that includes a full description of any highly exposed or highly susceptible subpopulations evaluated (e.g., children, the elderly). The questions the assessment attempts to answer are to be discussed and the assessment endpoints are to be well defined.
2. The methods used for the analysis (including all models used, all data upon which the assessment is based, and all assumptions that have a significant impact upon the results) are to be documented and easily located in the report. This documentation is to include a discussion of the degree to which data used are representative of the population under study. Also, this documentation is to include the names of models and software used to generate the analysis. Sufficient information is to be provided to allow the result of the analysis to be independently reproduced.
3. The results of the sensitivity analysis are to be presented and discussed in the report. Probabilistic techniques should be applied to the pathways and factors of importance to the assessment, as determined by sensitivity analyses or other basic requirements of the assessment.
4. The presence or absence of moderate to strong correlations or dependencies between the input variables is to be discussed and accounted for in the analysis, along with the effects these have on the output distribution.
5. Information for each input and output distribution is to be provided in the report. This includes tabular and/or graphical representations of the distributions (e.g., probability density function and cumulative distribution function plots) that indicate the location of any point estimate of interest (e.g., mean, median, 95th percentile). The selection of distributions is to be explained and justified. For both the input and output distributions, variability and uncertainty are to be differentiated where possible.
6. The numerical stability of the central tendency and the higher end (i.e., tail) of the output distributions are to be presented and discussed.
7. Calculations of exposures and risks using deterministic (e.g., point estimate) methods are to be reported if possible and/or appropriate. Providing these values will allow comparisons between the probabilistic analysis and past or screening level risk assessments. Further, deterministic estimates may be used to answer scenario specific questions and to facilitate risk communication. When comparisons are made, it is important to explain similarities and differences in the underlying data, assumptions, and models.
8. Since fixed exposure assumptions (e.g., exposure duration, body weight) are sometimes embedded in the toxicity metrics (e.g., Reference Doses, Reference Concentrations, unit cancer risk factors), the exposure estimates from the probabilistic output distribution are to be aligned with the toxicity metric.^[32]

³² Note that condition of acceptance Number 8 is not relevant for MRA because defaults that apply to all MRAs have not yet been developed.

5.5 Risk Description

As indicated above, the second component of risk characterization is the risk description. The purpose of the risk description is to summarize the event of interest according to its nature, severity, and consequences. In this regard, it is the risk description that is the synthesis of all of the previous components conducted within scope of the assessment. The risk description should:

- *Summarize the key issues and conclusions:* Information from the exposure and health effects components of the risk assessment should be integrated to arrive at conclusions for the microbial risk assessment. The key issues that impact the results should be summarized and put into context.
- *Discuss uncertainty, variability, and confidence in the results:* A candid and open discussion of the uncertainty in the overall assessment, in each of its components, and related estimates of risk is critical to a full characterization of risk. Uncertainty and sensitivity analysis are often conducted to develop the information needed for this purpose.
- *Address items in problem formulation:* The risk characterization should include a discussion of whether the assessment adequately addresses the questions delineated during problem formulation. For example, the risk management options defined during problem formulation could be used to develop risk estimates with and without proposed control measures. A discussion of the most sensitive variables (sensitivity analysis), or the variables with the largest contribution to the overall uncertainty in the risk estimate, may provide risk managers with insights that can be used for future resource allocation for developing risk mitigation strategies. As new data become available or as risk managers ask new questions, the problem formulation and analysis phases can be revisited and the assessment revised as needed and appropriate. Discussions of variability, uncertainty, and identified gaps in the knowledgebase should be reiterated from the discussions presented in the problem formulation.
- *Ensure transparency, clarity, consistency, and reasonableness (TCCR):* The importance of TCCR in all stages of an MRA has been highlighted previously. TCCR is particularly relevant for risk characterization because a risk assessment is often judged by the extent to which the risk characterization achieves the principles of TCCR.
- *Summarize assumptions:* A summary of key assumptions should be provided as a fundamental component of the risk description. If assumptions are unchanged and adequately described in the problem formulation documentation, it is not necessary to reiterate the assumptions. However, in the spirit of ensuring TCCR, a summary of assumptions in the risk description can be valuable.
- *Describe strengths and limitations:* As the assumptions, approaches, and conclusions of the risk assessment are presented, the strengths and limitations should be discussed. The assessment of data quality should be part of a risk characterization. Whenever possible, the data that are used should be both relevant and of high quality; however, it should be understood that the quality of available information will vary substantially. A candid discussion of the quality of the data employed should be provided, including how the data quality pertains to variability and uncertainty. Sufficient detail should be provided so that the assessment could be duplicated by others.

- *Discuss how the specific risk and its context compares with similar risks:* A discussion (at least in a qualitative manner) of how a specific risk compares with similar risks and discussion of the plausibility of the risk scenarios (ground truthing) is valuable for TCCR. This may be accomplished by comparisons with other pollutants or situations on which the Agency has already decided to act, or other situations that may be relevant. The discussion should highlight the limitations of such comparisons as well as the relevance of the comparisons

References

This reference list includes references cited in the main document as well as appendices.

Acheson, D.W.K., and Luccioli, S. (2004) Microbial-gut interactions in health and disease. Mucosal immune responses. *Best Practice & Research Clinical Gastroenterology* 18(2):387-404.

Airoldi M. and Morton A. (2009) Adjusting life for quality or disability: stylistic difference or substantial dispute? *Health Economics* In Press.

Aitken, C., and Jeffries, D.J. (2001) Nosocomial spread of viral disease. *Clinical Microbiology Reviews* 14(3):528-546.

Akaike, H. (1981) Likelihood of a model and information criteria, *Journal of Econometrics* 16:3-14.

Allen, L.J.S., and Allen, E.A. (2003) A comparison of three different stochastic population models with regard to persistence time. *Theoretical Population Biology* 64:439-449.

Amvrosieva, T.V., Titov, L.P., Mulders, M., Hovi, T., Dyakonova, O.V., Votyakov, V.I., Kvacheva, Z.B., Eremin, V.F., Sharko, R.M., Orlova, S.V., Kazinets, O.N., and Bogush, Z.F. (2001) Viral water contamination as the cause of aseptic meningitis outbreak in Belarus. *Central European Journal of Public Health* 9(3):154-157.

Anderson, R.M., and May, R. (1991) *Infectious Diseases of Humans: Dynamics and Control*. New York: Oxford University Press.

Armstrong, T.W., and Haas, C.N. (2008) Legionnaires' disease: evaluation of a quantitative microbial risk assessment model. *Journal of Water and Health* 6(2):149-166.

Bailey, N.T.J. (1964) *The Elements of Stochastic Processes with Application to the Natural Sciences*. New York: John Wiley and Sons.

Begier, E.M., Oberste, M.S., Landry, M.L., Brennan, T., Mlynarski, D., Mshar, P.A., Frenette, K., Rabatsky-Ehr, T., Purviance, K., Nepaul, A., Nix, W.A., Pallansch, M.A., Ferguson, D., Cartter, M.L., and Hadler, J.L. (2008) An outbreak of concurrent echovirus 30 and coxsackievirus A1 infections associated with sea swimming among a group of travelers to Mexico. *Clinical and Infectious Diseases* 47(5):616-623.

Bell, J.F., Owens, C.R., and Larson, C.L. (1955) Virulence of *Bacterium tularensis*. I. A study of the virulence of *Bacterium tularensis* in mice, guinea pigs, and rabbits. *Journal of Infectious Diseases* 97(2):162-167.

- Bieber, D., Ramer, S.W., Wu, C.Y., Murray, W.J., Tobe, T., Fernandez, R., and Schoolnik, G.K. (1998), Type IV pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli*. *Science* 280(5372):2114-2118.
- Blaser, M.J., and Kirschner, D. (1999) Dynamics of *Helicobacter pylori* colonization in relation to the host response. *Proceedings of the National Academy of Sciences (USA)* 96(15):8359-8364.
- Blaser, M.J., and Kirschner, D. (2007) The equilibria that allow bacterial persistence in human hosts. *Nature* 449(7164):843-849.
- Boerlijst, M.C., Bonhoeffer, S., and Nowak, M.A. (1996) Viral quasi-species and recombination. *Proceedings: Biological Sciences* 263(1376):1577-1584.
- Boehm, A.B., Grant, S.B., Kim, J.H., Mowbray, S.L., McGee, C.D., Clark, C.D., Foley, D.M., and Wellman, D.E. (2002) Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environmental Science & Technology* 36(18):3885-3892.
- Boehm, A.B. (2007) Enterococci concentrations in diverse coastal environments exhibit extreme variability. *Environmental Science & Technology* 41:8227-8232.
- Bollaerts, K., Aerts, M., Faes, C., Grijspeerdt, K., Dewulf, J., and Mintiens, K. (2008) Human salmonellosis: estimation of dose-illness from outbreak data. *Risk Analysis* 28(2):427-440.
- Bogosian, B.J., and Bourneuf, E.V. (2001) A matter of bacteria life and death. *EMBO Reports* 2(9):770-774.
- Brookmeyer, R., Johnson, E., and Barry, S. (2005) Modelling the incubation period of anthrax. *Statistics in Medicine* 24:531-542.
- Brown, M.R., and Barker, J. (1999) Unexplored reservoirs of pathogenic bacteria: protozoa and biofilms. *Trends in Microbiology* 7(1):46-50.
- Brynstad, S., Braute, L., Lubber, P., and Bartelt, E. (2008) Quantitative microbiological risk assessment of campylobacteriosis cases in the German population due to consumption of chicken prepared in homes. *International Journal of Risk Assessment and Management* 8(3):194-213.
- Buchanan, R.L., Lammerding, A.M., Clarke, I.R., van Schothorst, M., and Roberts, T.A. (1998) Potential application of risk assessment techniques to microbiological issues related to international trade in food and food products. *Journal of Food Protection* 61:1075-1086.
- Buchanan, R.L., Smith, J.L., and Long, W. (2000) Microbial risk assessment: dose-response relations and risk characterization. *International Journal of Food Microbiology* 58:159-172.
- Bukhari, Z., Hargy, T.M., Bolton, J.R., Dussert, B., and Clancy, J.L. (1999) Medium-pressure UV for oocyst inactivation. *Journal of the American Water Works Association* 91:86-94.

CAC (Codex Alimentarius Commission) (1999) Draft Principles and Guidelines for the Conduct of Microbiological Risk Assessment. (Step 8 of Codex elaboration) CAC/GL-30(1999). ftp://ftp.fao.org/codex/standard/en/CXG_030e.pdf

CAC (2004) Codex Alimentarius Commission - Procedural Manual Fourteenth Edition, Section III-Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius. http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y5817e/y5817e00.htm

CAC (2007) Draft Principles and Guidelines for the Conduct of Microbiological Risk Management. (Step 8 of Codex elaboration) Alinorm 07/30/13. Report of 38th session of the Codex Committee on Food Hygiene.

Carbone, K.M., Luftig, R.B., and Buckley, M.R.. (2005) Microbial Triggers of Chronic Human Illness. American Academy of Microbiology. Washington, DC.

CDC (U.S. Centers for Disease Control and Prevention) (1992) Principles of Epidemiology, Self-Study Course 3030-G, Second Edition <http://www.uic.edu/sph/prepare/courses/ph490/resources/epiintro.pdf>
<http://www.uic.edu/sph/prepare/courses/ph490/resources/epilesson01.pdf>

CDC (2004) Centers for Disease Control and Prevention Surveillance for Waterborne-Disease Outbreaks associated with recreational water - United States, 2001-2002 and, Surveillance for Waterborne-Disease Outbreaks associated with drinking water - United States, 2001-2002. *Surveillance Summaries*, October 22, 2004. *Morbidity and Mortality Weekly Reports* 53(SS-8).

Chappell, C.L., Okhuysen, P.C., Sterling, C.R., Wang, C., Jakubowski, W., and DuPont, H.L. (1999) Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *American Journal of Tropical Medicine and Hygiene* 60(1):157-164.

Chick, S.E., Koopman, J.S., Soorapanth, S., and Brown, M.E. (2001) Infection transmission system models for microbial risk assessment. *The Science of the Total Environment* 274:197-207.

Clancy, J.L., Bukhari, Z., Hargy, T.M., Bolton, J.R., Dussert, B.W., and Marshall, M.M. (2000) Using UV to inactivate *Cryptosporidium parvum* by ultraviolet radiation. *Journal of the American Water Works Association* 92:97-104.

Clough, H.E., Clancy, D., O'Neill, P.D., and French, N.P. (2003). Bayesian methods for estimating pathogen prevalence within groups of animals from faecal-pat sampling. *Preventive Veterinary Medicine* 58(3-4):145-69.

Colford, J.M., Jr., Wade, T.J., Sandhu, S.K., Wright, C.C., Lee, S., Shaw, S., Fox, K., Burns, S., Benker, A., Brookhart, M.A., van der Laan, M., and Levy, D.A. (2005) A randomized, controlled trial of in-home drinking water intervention to reduce gastrointestinal illness. *American Journal of Epidemiology* 161(5):472-482.

- Coleman, M., and Marks, H. (1998) Topics in dose-response modeling. *Journal of Food Protection* 61: 1550-1559.
- Coleman, M., and Marks, H. (2000) Mechanistic Modeling of Salmonellosis. *Quantitative Microbiology* 2: 227-247.
- Coleman, M.E., Marks, H.M., Golden, N.J., and Latimer, H.K. (2004) Discerning strain effects in microbial dose-response data. *Journal of Toxicology and Environmental Health-Part A-Current Issues* 67:667-685.
- Colwell, R.R., Huq, A., Islam, M.S., Aziz, K.M.A., Yunus, M., Khan, N.H., Mahmud, A., Sack, R.B., Nair, G.B., Chakraborty, J., Sack, D.A., and Russek-Cohen, E. (2003) Reduction of cholera in Bangladeshi villages by simple filtration. *Proceedings of the National Academy of Sciences (USA)* 100(3):1051-1055.
- Couch, R.B., Cate, T.R., Gerone, P.J., Fleet, W.F., Lang, D.J., Griffith, W.R., and Knight, V. (1965) Production of illness with a small-particle aerosol of coxsackie A21. *Journal of Clinical Investigation* 44:535-542.
- Couch, R. B., Cate, T.R., Douglas, R.G. Jr., Gerone, P.J., and Knight, V. (1966) Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriological Reviews* 30(3):517-529.
- Covacci, A., and Rappuoli, R. (1998) *Helicobacter pylori*: Molecular evolution of a bacterial quasi-species. *Current Opinion in Microbiology* 1(1):96-102.
- Crabtree, K.D., Gerba, C.P., Rose, J.B., and Haas, C.N. (1997) Waterborne adenovirus: a risk assessment. *Water Science and Technology* 35(11-12):1-6.
- Craik, S.A., Weldon, D., Finch, G.R., Bolton, J.R., and Belosevic, M. (2001) Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Water Research* 35(6):1387-1398.
- Crainiceanu, C.M., Stedinger, J.R., Ruppert, D., and Behr, C.T. (2003) Modeling the United States national distribution of waterborne pathogen concentrations with application to *Cryptosporidium parvum*. *Water Resources Research* 39(9):SWC 2-1.
- Crépet, A., Stahl, V., and Carlin, F. 2009. Development of a hierarchical Bayesian model to estimate the growth parameters of *Listeria monocytogenes* in minimally processed fresh leafy salads. *International Journal of Food Microbiology* 131(2-3):112-9.
- Doyle, T.J., Glynn, K.M., and Gorsealose, S.L. (2002) Completeness of notifiable infectious disease reporting in the US: an analytical literature review. *American Journal of Epidemiology* 155:866-874.

Dudley, R.H., Hekimian, K.K., and Mechalas, B.J. (1976) A scientific basis for determining recreational water quality criteria. *Journal - Water Pollution Control Federation* 48:2661-2677.

Dufour, A., Evans, O., Behymer, T., and Cantu, R. (2006) Water ingestion during swimming activities in a pool: a pilot study. *Journal of Water and Health* 4: 425-430.

DuPont, H., Chappell, C., Sterling, C., Okhuysen, P., Rose, J., and Jakubowski, W. (1995) The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* 332:855-859.

Eckmann, L. (2006) Sensor molecules in intestinal innate immunity against bacterial infections. *Current Opinion in Gastroenterology* 22(2):95-101.

Effler, P., Jeong, M-C., Kimura, A., Nakata, M., Burr, R., Cremer, E., and Slutsker, L. (2001) Sporadic *Campylobacter jejuni* infections in Hawaii: associations with prior antibiotic use and commercially prepared chicken. *Journal of Infectious Diseases* 83:1152-1155.

Eisenberg, J.N., Seto, E.Y.W., Olivieri, A.W., and Spear, R.C. (1996) Quantifying water pathogen risk in an epidemiological framework. *Risk Analysis* 16:549-563.

Eisenberg, J.N., Seto, E.Y.W., Colford, J.M., Olivieri, A.W., and Spear, R.C. (1998) An analysis of the Milwaukee cryptosporidiosis outbreak based on a dynamic model of the infection process. *Epidemiology* 9:255-263.

Eisenberg, J.N., Brookhart, M.A., Rice, G., Brown, M., and Colford, J.M., Jr. (2002) Disease transmission models for public health decision making: analysis of epidemic and endemic conditions caused by waterborne pathogens. *Environmental Health Perspectives* 110(8):783-790.

Eisenberg, J.N.S., Lewis, B.L., Porco, T.C., Hubbard, A.H., and Colford, J.M. (2003) Bias due to secondary transmission in estimation of attributable risk from intervention trials. *Epidemiology* 14(4):442-450.

Eisenberg, J.N.S., Soller, J.A., Scott, J., Eisenberg, D.M., and Colford, J.M. (2004) A dynamic model to assess microbial health risks associated with beneficial uses of biosolids. *Risk Analysis* 24:221-236.

Eisenberg, J.N., Moore, K., Soller, J.A., Eisenberg, D., and Colford, J.M., Jr. (2008) Microbial risk assessment framework for exposure to amended sludge projects. *Environmental Health Perspectives* 116:727-733.

Englehardt, J.D. (2004) Predictive Bayesian dose-response assessment for appraising absolute health risk from available information. *Human and Ecological Risk Assessment* 10(1):69-78.

Englehardt, J.D., and Swartout, J. (2004) Predictive population dose-response assessment for *Cryptosporidium parvum*: Infection endpoint. *Journal of Toxicology and Environmental Health-Part A-Current Issues* 67(8-10):651-666.

Englehardt, J.D, and Swartout, J. (2006), Predictive Bayesian microbial dose-response assessment based on suggested self-organization in primary illness response: *Cryptosporidium parvum*. *Risk Analysis* 26(2):543-554.

Englehardt, J.D., and Swartout, J. (2008) Development and Evaluation of Novel Dose-Response Models for Use in Microbial Risk Assessment, Technical report, National Center for Environmental Assessment Office of Research and Development U.S. Environmental Protection Agency. EPA/600/R-08/033.

EPA (U.S. Environmental Protection Agency) (1986a) Ambient Water Quality Criteria for Bacteria – 1986. Office of Water Regulations and Standards. Washington, DC. EPA/440/5-84/002.

EPA (1986b) Quality Criteria for Water 1986. EPA 440/5-86-001.
<http://www.epa.gov/waterscience/criteria/goldbook.pdf>

EPA (1989) Risk Assessment Guidance for Superfund. EPA-540/1-89/002. Washington, DC.
<http://www.epa.gov/oswer/riskassessment/ragsa/index.htm>

EPA (1992) Framework for Ecological Risk Assessment. Washington, DC. EPA/630/R-92/001.

EPA (1994) Peer Review and Peer Involvement at the U.S. Environmental Protection Agency. Washington, DC. <http://epa.gov/osa/spc/html/perevmem.htm>

EPA (1995a) Guidance for Risk Characterization. U.S. Environmental Protection Agency, Science Policy Council. Washington, DC.
<http://www.epa.gov/OSA/spc/pdfs/rcguide.pdf>

EPA (1995b) A Guide to the Biosolids Risk Assessment for the EPA Part 503 Rule. Office of Wastewater Management. Washington, DC. EPA/832-B-93-005.

EPA (1995c) The EPA's Environmental Justice Strategy.
http://www.epa.gov/compliance/resources/policies/ej/ej_strategy_1995.pdf

EPA (1997a) Exposure Factors Handbook. Office of Research and Development, National Center for Environmental Assessment. Washington, DC. EPA/600/P-95/002Fa.

EPA (1997b) Guiding Principles for Monte Carlo Analysis. EPA/630/R-97/001.

EPA (1998) Guidelines for Ecological Risk Assessment. May 14, 1998, *Federal Register* 63(93):26846-26924. EPA/630/R-95/002.F.

EPA (2000a) EPA Science Policy Council Peer Review Handbook. Washington, DC. EPA-100-B-00-001.

EPA (2000b) Science Policy Council Risk Characterization Handbook. Washington, DC. EPA-100-B-00-002 <http://www.epa.gov/osa/spc/pdfs/rchandbk.pdf>

EPA (2000c) Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. EPA-822-B-00-004.
<http://www.epa.gov/waterscience/humanhealth/method/complete.pdf>

EPA (2000d) Report to Congress EPA Studies on Sensitive Subpopulations and Drinking Water Contaminants. EPA 815-R-00-015.
http://www.epa.gov/safewater/standard/rtc_sensubpops.pdf

EPA (2001) Protocol for Developing Pathogen TMDLs. EPA 841-R-00-002.
http://www.epa.gov/owow/tmdl/pathogen_all.pdf

EPA (2002a) Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of Information Disseminated by the Environmental Protection Agency. EPA/260R-02-008.
http://www.epa.gov/quality/informationguidelines/documents/EPA_InfoQualityGuidelines.pdf

EPA (2002b) Lessons Learned on Planning and Scoping for Environmental Risk Assessments, EPA Science Policy Council. Washington, DC.
<http://epa.gov/osp/spc/handbook.pdf>

EPA (2002c) Microbiological Risk Assessment Framework Workshop Tools, Methods, and Approaches [Prepared by ICF Consulting, Inc.].

EPA (2002d) Child-Specific Exposure Factors Handbook. EPA-600-P-00-002B.

EPA (2002e) National Primary Drinking Water Regulations: Long Term 1 Enhanced Surface Water Treatment Rule. 40CFR Parts 9, 141 and 142, and *Federal Register* 67(9), January 14, 2002.
<http://www.epa.gov/safewater/mdbp/lt1eswtr.html>

EPA (2003a) National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule Proposal. *Federal Register* 68:154.

EPA (2003b) Economic Analysis for the Long Term 2 Enhanced Surface Water Treatment Rule Proposal.

EPA (2003c) Microbiological Risk Assessment Framework: Problem Formulation Workshop [Prepared by ICF Consulting, Inc.]

EPA (2003d) Movement and Longevity of Viruses in the Subsurface. National Risk Management Research Laboratory. EPA/540/S-03/500.
<http://www.epa.gov/ada/download/issue/540S03500.pdf>

EPA. (2003e). “Integrated Risk Information, Glossary of IRIS terms.” U.S. Environmental Protection Agency. <http://www.epa.gov/iris/gloss8.htm>

EPA (2003f) Framework for Cumulative Risk Assessment. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA/630/P-02/001F. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=54944>

EPA (2003g) Public Involvement Policy of the U.S. Environmental Protection Agency. EPA 233-B-03-002. <http://www.epa.gov/publicinvolvement/pdf/policy2003.pdf>

EPA (2004a) An Expert Judgment Assessment of the Concentration-Response Relationship Between PM_{2.5} Exposure and Mortality. Office of Air Quality Planning and Standards (OAQPS) Report and Peer Review of the Report.
Report: <http://www.epa.gov/ttn/ecas/regdata/Benefits/pmexpert.pdf>
Peer review of the report: http://www.epa.gov/ttn/ecas/regdata/Benefits/memo_7.30.04.pdf

EPA. (2004b). Air Toxics Risk Assessment Reference Library: Volume 1 Technical Resource Manual. U.S. Environmental Protection Agency. EPA-453-K-04-001A.
http://www.epa.gov/ttn/fera/risk_atra_main.html
http://www.epa.gov/ttn/fera/data/risk/vol_1/glossary.pdf

EPA (2004c) Developing dynamic infection transmission models for microbial risk assessment applications, EPA-NCEA-C-1463.

EPA (2004d) Risk Assessment Principles and Practices, Office of the Science Advisor Staff Paper. EPA/100/B-04/001. <http://www.epa.gov/OSA/pdfs/ratf-final.pdf>

EPA. (2005a). Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001B.
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283>

EPA (2005b) Guidance on selecting age groups for monitoring and assessing childhood exposures to environmental contaminants. National Center for Environmental Assessment, Washington, DC. EPA/630/P-03/003F.

EPA (2005c) Rule to Reduce Interstate Transport of Fine Particulate Matter and Ozone (Clean Air Interstate Rule); Revisions to Acid Rain Program; Revisions to the NOX SIP Call; Final Rule. Federal Register 70(91). May 12, 2005. Pages 25162-25405.
<http://www.epa.gov/air/interstateairquality/rule.html>

EPA (2006a) National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule - Final. *Federal Register* 71(3). Including Economic Analysis and Appendices A-U. <http://www.epa.gov/safewater/disinfection/lt2/regulations.html>

EPA (2006b) National primary drinking water regulations: ground water rule. 40CFR Parts 9, 141 and 142, *Federal Register* 71(216), November 8, 2006. Including Economic Analysis and Appendices.

<http://www.epa.gov/safewater/disinfection/gwr/>

EPA (2007a) Thesaurus of Terms Used in Microbiological Risk Assessment. EPA Office of Water.

<http://www.epa.gov/waterscience/criteria/humanhealth/microbial/thesaurus/index.html>

<http://www.epa.gov/waterscience/criteria/humanhealth/microbial/thesaurus/T7.html>

EPA (2007b) Compendium of Prior and Current Microbial Risk Assessment Methods for Use as a Basis for the Selection, Development, and Testing of a Preliminary Microbial Risk Assessment Framework. National Homeland Security Research Center. EPA/600/R-07/129.

<http://www.epa.gov/NHSRC/pubs/600r07129.pdf>

EO (1994) Executive Order 12898—Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations.

<http://www.epa.gov/fedrgstr/eo/eo12898.htm>

EO (1997) Executive Order 13045—Protection of Children from Environmental Health Risks and Safety Risks. *Federal Register* 62(78):19883-19888.

Fankhauser, R.L., Noel, J.S., Monroe, S.S., Ando, T., and Glass, R.I. (1998) Molecular epidemiology of “Norwalk-like viruses” in outbreaks of gastroenteritis in the United States. *Journal of Infectious Diseases* 178:1571-1578.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) (2003) Microbiological Risk Assessment Series, No. 3: Hazard Characterization for Pathogens in Food and Water, Guidelines.

<http://whqlibdoc.who.int/publications/2003/9241562374.pdf>

Farber, J.M., Ross, W.H., Harwig, J. (1996). Health risk assessment of *Listeria monocytogenes* in Canada. *International Journal of Food Microbiology* 30(1-2):145-156.

Faruque, S.M., Biswas, K., Udden, S.M.N., Ahmad, Q.S., Sack, D.A., Nair, G.B., and Mekalanos, J.J. (2006) Transmissibility of cholera: in vivo-formed biofilms and their relationship to infectivity and persistence in the environment. *Proceedings of the National Academy of Sciences (USA)* 103(16):6350-6355.

Fazil, A., Paoli, G., Lammerding, A.M., Davidson, V., Hrudey, S., Isaac-Renton, J., and Griffiths, M. (2005) Microbial Risk Assessment as a Foundation for Informed Decision-Making: A Needs, Gaps and Opportunities Assessment (NGOA) for Microbial Risk Assessment in Food and Water. Public Health Agency of Canada.

<http://www.uoguelph.ca/crifs/NGOA/Finalupdates/NGOAFinalreport.pdf>

FDA (U.S. Food and Drug Administration) (2001) Draft Risk Assessment on the Public Health Impact of *Vibrio parahaemolyticus* in Raw Molluscan Shellfish. Center for Food Safety and Applied Nutrition. <http://www.cfsan.fda.gov/~dms/vprisk4.html>

FDA (2002) Initiation and Conduct of all “Major” Risk Assessments Within a Risk Analysis Framework. A Report by the Center for Food Safety and Applied Nutrition Risk Analysis Working Group. <http://www.cfsan.fda.gov/~dms/rafw-toc.html>

FDA/USDA (U.S. Department of Agriculture) (2003) Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. Rockville, MD. <http://www.foodsafety.gov/~dms/lmr2-toc.html>

Forchielli, M.L., and Walker, W.A. (2005) The role of gut-associated lymphoid tissues and mucosal defence. *British Journal of Nutrition* 93 Suppl 1:S41-S48.

Frey, H.C., Mokhtari, H., and Zheng, J. (2004). Recommended Practice Regarding Selection, Application, and Interpretation of Sensitivity Analysis Methods Applied to Food Safety Process Risk Models. Prepared for the Office of Risk Assessment and Cost-Benefit Analysis, U.S. Department of Agriculture.

<http://www.ce.ncsu.edu/risk/Phase3Final.pdf>

Frost, F.J., Roberts, M., Kunde, T.R., Craun, G., Tollestrup, K., Harter, L., and Muller, T. (2005) How clean must our drinking water be: The importance of protective immunity. *Journal of Infectious Diseases* 191:809-814.

Fuhs, O.W. (1975) A probabilistic model of bathing beach safety. *Science of the Total Environment* 4:165-175.

Gale, P. (2003) Using event trees to quantify pathogen levels on root crops from land application of treated sewage sludge. *Journal of Applied Microbiology* 94:35-47.

Gale, P. (2005) Land application of treated sewage sludge: Quantifying pathogen risks from consumption of crops. *Journal of Applied Microbiology* 98:380-396.

Gerba, C.P. (2006) Enteroviruses and Parechoviruses. In: *Waterborne Pathogens*, 2nd ed., Denver, CO: American Water Works Association. Denver.

Gerba, C., Yates, M., Yates, S., and Hurst, C., eds. (1991) Quantitation of factors controlling viral and microbial transport in the subsurface. In: *Modeling the Environmental Fate of Microorganisms*, Washington, DC: American Society for Microbiology.

Gerba, C. P.; Rose, J. B. & Haas, C. N. (1996a) Sensitive populations: who is at the greatest risk? *International Journal of Food Microbiology* 30(1-2):113-123.

Gerba, C.P., Rose, J.B., Haas, C.N., and Crabtree, K.D. (1996b) Waterborne rotavirus: a risk assessment. *Water Research* 30:2929-2940.

Gerba, C.P., Henze, M., Loosdrecht, C.M., Ekman, G.A., and Brdjanovic, D., eds. (2008), Pathogen removal. In: *Biological Wastewater Treatment, Modeling and Design*, IWA Publishing, London.

Gilks, W., Richardson, S., and Spiegelhalter D.J. (eds.) (1996) *Markov Chain Monte Carlo in Practice*. London, UK: Chapman and Hall.

Gilks, W.R. and Wild, P. (1992). Adaptive rejection sampling for Gibbs sampling. *Applied Statistics* 41:337-48.

Girschick, H.J., Guilherme, L., Inman, R.D., Latsch, K., Rihl, M., Sherer, Y., Shoenfeld, Y., Zeidler, H., Arienti, S., and Doria, A. (2008) Bacterial triggers and autoimmune rheumatic diseases. *Clinical and Experimental Rheumatology* 26(1 Suppl 48):S12-S17.

Gold, M.R., Stevenson, D., and Fryback, D.G. (2002) HALYs and QALYs and DALYs, oh my: similarities and differences in summary measures of population health. *Annual Review Public Health* 23:115-134.

Gordis, L. (2000) *Epidemiology*. 2nd ed. Philadelphia, PA: W.B. Saunders Company.

Greenberg, H., Valdesuso, J., Kapikian, A., Chanock, R., Wyatt, R., Szmuness, W., Larrick, J., Kaplan, J., Gilman, R.H., and Sack, D.A. (1979) Prevalence of antibody to the Norwalk virus in various countries. *Infection and Immunity* 26:270-273.

Gronewold, A.D., Borsuk, M.E., Wolpert, R.L., and Reckhow, K.H. (2008). An assessment of fecal indicator bacteria-based water quality standards. *Environmental Science and Technology* 42(13):4676-82.

Gronewold, A.D., Qian, S.S., Wolpert, R.L., and Reckhow, K.H. (2009). Calibrating and validating bacterial water quality models: a Bayesian approach. *Water Research* 43:2688-2698.

Gutting, B.W., Channel, S.R., Berger, A.E., Gearhart, J.M., Andrews, G.A., and Sherwood, R.L. (2008) Mathematically modeling inhalation anthrax. *Microbe* 3(2):78-85.

Haas, C.N. (1983) Effect of effluent disinfection on risks of viral disease transmission via recreational water exposure. *Journal - Water Pollution Control Federation* 55:1111-1116.

- Haas, C.N., Crockett, C.S., Rose, J.B., Gerba, C.P., and Fazil, A.M. (1996) Assessing the risks posed by oocysts in drinking water. *Journal of the American Water Works Association* 88(9):131-136.
- Haas, C.N., Rose, J., and Gerba, C.P. (1999) Quantitative Microbial Risk Assessment. New York: Wiley.
- Harp, J.A. (2003) *Cryptosporidium* and host resistance: historical perspective and some novel approaches. *Animal Health Research Reviews* 4(1):53-62.
- Hamer, W.H. (1906) Endemic disease in England. *Lancet* 1:733-739.
- Hastings, W.K. (1970) Monte Carlo sampling methods using Markov Chains and their applications. *Biometrika* 57:97-109.
- Hauri, A.M., Schimmelpfennig, M., Walter-Domes, M., Letz, A., Diedrich, S., Lopez-Pila, J., and Schreier, E. (2005) An outbreak of viral meningitis associated with a public swimming pond. *Epidemiology and Infection* 133(2):291-298.
- Hethcote, H. (1976) Qualitative analyses of communicable disease models. *Mathematical Biosciences* 28:335-356.
- Hethcote, H.W. (2000) The mathematics of infectious diseases. *SIAM Review* 42:599-653.
- Holcomb, D.L., Smith, M.A., Ware, G.O., Hung, Y.C., Brackett, R.E., and Doyle, M.P. (1999) Comparison of six dose-response models for use with food-borne pathogens. *Risk Analysis* 19(6):1091-1100.
- Holdenfried, R. and Quan, S.F. (1956) Susceptibility of New Mexico rodents to experimental plague. *Public Health Reports* 71(10):979-984.
- Hopkins, R.S., Gaspard, G.B., Williams, F.P., Karlin, R.J., Cukor, K.G., and Blacklow, N.R. (1984) A community waterborne gastroenteritis outbreak: evidence for rotavirus as the agent. *American Journal of Public Health* 74:263-265.
- ILSI (International Life Sciences Institute) Risk Science Institute Pathogen Risk Assessment Working Group (1996) A conceptual framework for assessment of the risks of human disease following exposure to waterborne pathogens. *Risk Analysis* 16:841-848.
- ILSI Risk Science Institute. (2000) Revised Framework for Microbial Risk Assessment. Washington, DC. <http://www.ilsilife.org/file/mrabook.pdf>
- Jaidane, H., and Hober, D. (2008) Role of coxsackievirus B4 in the pathogenesis of type 1 diabetes. *Diabetes & Metabolism* 34(6 Pt 1):537-548.

- Jameel, S. (1999) Molecular biology and pathogenesis of hepatitis E virus. *Expert Reviews in Molecular Medicine* (6 December):1-16.
<http://www-ermm.cbcu.cam.ac.uk/99001271h.htm>
- King, A.A., Ionides, E.L., Pascual, M., and Bouma, M.J. (2008) Inapparent infections and cholera dynamics. *Nature* 454:877-880.
- Kodell, R.L., Kang, S-H., and Chen, J.J. (2002) Statistical models of health risk due to microbial contamination of foods. *Environmental and Ecological Statistics* 9:259-271.
- Koopman, J.S., Monto, A.S., and Longini, I.M., Jr. (1989) The Tecumseh Study XVI: Family and community sources of rotavirus infection. *American Journal of Epidemiology* 130(4):760-768.
- Koopman, J.S., Chick, S.E., Simon, C.P., Riolo, C.S., and Jacquez, G. (2002) Stochastic effects on endemic infection levels of disseminating versus local contacts. *Mathematical Biosciences* 180:49-71.
- Last, J.M. (ed.) (1995) *A Dictionary of Epidemiology*, 3rd ed. New York: Oxford University Press.
- Latimer, H.K., Jaykus, L-A., Morales, R.A., Cowen, P., and Crawford-Brown, D. (2001) A weighted composite dose response model for human salmonellosis. *Risk Analysis* 21(2):295-306.
- Lau, H.Y., and Ashbolt, N.J. (2009) The role of biofilms and protozoa in Legionella pathogenesis: implications for drinking water. *Journal of Applied Microbiology* In Press.
- Levin, B.R., and Antia, R. (2001) Why we don't get sick: the within-host population dynamics of bacterial infections. *Science* 292(5519):1112-1115.
- Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, L., Stewart, P., LePendu, J., and Baric, R. (2003) Human susceptibility and resistance to Norwalk virus infection. *Nature Medicine* 9(5):548-553.
- Lindesmith, L., Moe, C., LePendu, J., Frelinger, J.A., Treanor, J., and Baric, R.S. (2005) Cellular and humoral immunity following Snow Mountain virus challenge. *Journal of Virology* 79:2900-2909.
- Loewe, L., Textor, V., and Scherer, S. (2003) High deleterious genomic mutation rate in stationary phase of *Escherichia coli*. *Science* 302:1558-1559.
- Lyons, C.R., and Wu, T.H. (2007) Animal models of *Francisella tularensis* infection. *Annals of the New York Academy of Science* 1105:238-265.

- MacKenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B., and Davis, J.P. (1994) A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine* 331(3):161-167.
- Makri, A., Modarres, E., and Parkin R. (2004). Cryptosporidiosis susceptibility and risk: A case study. *Risk Analysis* 24(1):209-220.
- Marino, S., Beretta, E., and Kirschner, D.E. (2007) The role of delays in innate and adaptive immunity to intracellular bacterial infection. *Mathematical Biosciences and Engineering* 4(2), 261-288.
- Marionneau, S., Ruvoen, N., Le Moullac-Vaidye, B., Clement, M., Cailleau-Thomas, A., Ruiz-Palacois, G., Huang, P., Jiang, X., and Le Pendu, J. (2002) Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* 122(7):1967-1977.
- McBride, G.B., Till, D., Ryan, T., Ball, A., Lewis, G., Palmer, S., and Weinstein, P. (2002) Freshwater Microbiology Research Programme. Pathogen Occurrence and Human Health Risk Assessment Analysis. Technical Publication, 93 pp., Ministry for the Environment, Wellington. <http://www.mfe.govt.nz/publications/water/freshwater-microbiology-nov02/freshwater-microbiology-nov02.pdf>
- Medema, G., and Smeets, P. (2004) The Interaction Between Quantitative Microbial Risk Assessment and Risk Management in the Water Safety Plan. Kiwa Water Research/Delft University. http://217.77.141.80/clueadeau/microrisk/uploads/interaction_in_water_safety_plan.pdf
- Mena, K., Gerba, C.P., Haas, C.N., and Rose, J.B. (2003) Risk assessment of waterborne coxsackievirus. *Journal of the American Water Works Association* 95:122-131.
- Messner, M.J., Chappell, C.L., and Okhuysen, P.C. (2001) Water risk assessment for *Cryptosporidium*: a hierarchical Bayesian analysis of human dose response data. *Water Research* 35(16):3934-3940.
- Moe, C.L., Frelinger, J.A., Heizer, W., and Stewart, P. (2002) Studies of the infectivity of Norwalk and Norwalk-like viruses. Submitted to EPA National Center for Environmental Research (#R826139). Washington, DC: EPA. http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/192/report/F
- Moon, H., Chen, J.J., Gaylor, D.W., and Kodell, R.L. (2004) A comparison of microbial dose-response models fitted to human data. *Regulatory Toxicology and Pharmacology* 40:177-184.

- Moon, H., Kim, H-J., Chen, J.J., and Kodell, R.L. (2005) Model averaging using the Kullback Information Criterion in estimating effective doses for microbial infection and illness. *Risk Analysis* 25(5):1147-1159.
- Morales, M.A.G., and Pozio, E. (2002) Humoral and cellular immunity against *Cryptosporidium* infection. *Current Drug Targets - Immune, Endocrine & Metabolic Disorders* 2(3):291-301.
- Morgan, M.G., and Henrion, M. (eds.) (1990) Uncertainty: A Guide to Dealing with Uncertainty in Quantitative Risk and Policy Analysis. New York, NY: Cambridge University Press.
- Morgan, U.M., Xiao, L., Sulaiman, I., Weber, R., Lal, A.A., Thomson, R.C., and Deplazes, P. (1999) Which genotypes/species of *Cryptosporidium* are humans susceptible to? *Journal of Eukaryotic Microbiology* 46(5):42S-43S.
- Namata, H., Aerts, M., Faes, C., and Teunis P. (2008) Model averaging in microbial risk assessment using fractional polynomials. *Risk Analysis* 28(4):891-905.
- Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., and Havelaar, A. (2009) A comparison of risk assessments on *Campylobacter* in broiler meat. *International Journal of Food Microbiology* 129(2):107-123.
- NCRP (National Committee on Radiation Programs) (1996) A Guide for Uncertainty Analysis in Dose and Risk Assessments Related to Environmental Contamination. NCRP, Scientific Committee 64-17, Washington, DC. NCRP Commentary No. 14 [as cited in EPA, 1997b, page 14].
- NRC (National Research Council) (1983) Risk Assessment in the Federal Government: Managing the Process. Washington, DC: National Academy Press.
- NRC (2004) Indicators for Waterborne Pathogens. Washington, DC: The National Academies Press.
- Okhuysen, P.C., Chappell, C.L., Crabb, J.H., Sterling, C.R., and DuPont, H.L. (1999) Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *Journal of Infectious Diseases* 180(4):1275-1281.
- OMB (Office of Management and Budget of the White House) (2004) Revised Information Quality Bulletin for Peer Review, April 2004.
http://www.whitehouse.gov/omb/inforeg/peer_review041404.pdf

Omenn, G.S., Kessler, A.C., Anderson, N.T., Chiu, P.Y., Doull, J., Goldstein B., Lederberg, J., McGuire, S., Rall, D., and Weldon, V.V. (1997) Framework for Environmental Health Risk Management/Risk Assessment and Risk Management in Regulatory Decision-Making: Final Report (2 vol.). Presidential/Congressional Commission on Risk Assessment and Risk Management.

<http://www.riskworld.com/Nreports/1997/risk-rpt/pdf/epajan.pdf>

<http://www.riskworld.com/Nreports/1997/risk-rpt/volume2/pdf/V2EPA.pdf>

Oscar, T.P. (2005) Validation of lag time and growth rate models for *Salmonella typhimurium*: acceptable prediction zone method. *Journal of Food Science* 70(2):M129-M137.

Ouchi, F. (2004) A Literature Review on the Use of Expert Opinion in Probabilistic Risk Analysis. World Bank Policy Research Working Paper (Report Number WPS3201).

<http://econ.worldbank.org>

Parkin, R.T., and Balbus, J.M. (2000) Variations in concepts of “susceptibility” in risk assessment. *Risk Analysis* 20:603-611.

Payment, P., and Riley, M.S. (2002) Resolving the Global Burden of Illness: A Call to Action. American Academy of Microbiology, Washington, DC.

Pinsky, P.F. (2000) Assessment of risks from long term exposure to waterborne pathogens. *Environmental and Ecological Statistics* 7:155-175.

Pouillot, R., Albert, I., Cornu, M., and Denis, J.B. 2003. Estimation of uncertainty and variability in bacterial growth using Bayesian inference. Application to *Listeria monocytogenes*. *International Journal of Food Microbiology* 81(2):87-104.

Prüss, A., Kay, D., Fewtrell, L., and Bartram, J. (2002) Estimating the burden of disease from water, sanitation and hygiene at a global level. *Environmental Health Perspectives* 110(5):537-542.

Quan, S. F., McManus, A.G., and von Fintel, H. (1956) Infectivity of tularemia applied to intact skin and ingested in drinking water. *Science* 123(3204):942-943.

Ramachandran, G. (2001). Retrospective exposure assessment using bayesian methods. *Annals of Occupational Hygiene* 45(8):651-667.

Rangel, J.M., Sparling, P.H., Crowe, C., Griffin, P.M., and Swerdlow, D.L. (2005) Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerging Infectious Diseases* 11: 603-609.

Ranta, J., Tuominen, P., and Majjala, R. (2005) Estimation of true *Salmonella* prevalence jointly in cattle herd and animal populations using Bayesian hierarchical modeling. *Risk Analysis* 25(1):23-37.

Riley, S., Fraser, C., Donnelly, C.A., Ghani, A.C., Abu-Raddad, L.J., Hedley, A.J., Leung, G.M., Ho, L.M., Lam, T.H., Thach, T.Q., Chau, P., Chan, K.P., Lo, S.V., Leung, P.Y., Tsang, T., Ho, W., Lee, K.H., Lau, E.M., Ferguson, N.M., and Anderson, R.M. (2003) Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions. *Science* 300:1961-1966.

Regli, S., Rose, J.B., Haas, C.N., and Gerba, C.P. (1991) Modeling the risk from *Giardia* and viruses in drinking-water. *Journal of the American Water Works Association* 83:76-84.

Rendtorff, R.C. (1954) The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *American Journal of Hygiene* 59:209-220.

Rendtorff, R.C., and Holt, C.J. (1954). The experimental transmission of human intestinal protozoan parasites. IV. Attempts to transmit *Entamoeba coli* and *Giardia lamblia* by water. *American Journal of Hygiene* 60:327-328.

Rice, G., Heberling, M.T., Rothermich, M., Wright, J.M., Murphy, P.A., Craun, M.F. and Craun, G.F. (2006) The role of disease burden measures in future estimates of endemic waterborne disease. *Journal of Water and Health* 4 Suppl 2:187-199.

Rogers, K.A., Rogers, A.B., Leav, B.A., Sanchez, A., Vannier, E., Uematsu, S., Akira, S., Golenbock, D., and Ward, H.D. (2006) MyD88-dependent pathways mediate resistance to *Cryptosporidium parvum* infection in mice. *Infection and Immunity* 74(1):549-556.

Rose, J.B., and Grimes, D.J. (2001). Reevaluation of Microbial Water Quality: Powerful New Tools for Detection and Risk Assessment. Washington, DC: American Academy of Microbiology.

Rose, J.B., Haas, C.N., and Regli, S. (1991) Risk assessment and control of waterborne giardiasis. *American Journal of Public Health* 81:709-713.

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

Rose, J.B., and Gerba, C.P. (1991) Use of risk assessment for development of microbial standards. *Water Science and Technology* 24:29-34.

Roseberry, A.M. and D.E. Burmaster. (1992) Lognormal distribution for water intake by children and adults. *Risk Analysis* 12:99-1004.

Ross, R. (1911) *The Prevention of Malaria*. London: Murray.

Sanaa, M., Bemrah, N., Meyer, S., Cerf, O., and Mohammed, H. (2000) Quantitative risk assessment related to microbial food contamination. *Revue D' Epidemiologie et De Sante Publique* 48(SUPP2):11-23.

- Sartwell, P.E. (1950) The distribution of incubation periods of infectious disease. *American Journal of Hygiene* 51:310-318.
- Snedecor, G.W., and Cochran, W.G. (1989). *Statistical Methods*, 8th Edition, Iowa State University Press.
- Soller, J.A., Eisenberg, J.N., and Olivieri, A.W. (1999) Evaluation of Pathogen Risk Assessment Framework. Oakland, CA: Eisenberg, Olivieri and Associates.
- Soller, J.A., Olivieri, A., Crook, J., Parkin, R., Spear, R., Tchobanoglous, G., and Eisenberg, J.N.S. (2003) Risk-based approach to evaluate the public health benefit of additional wastewater treatment. *Environmental Science & Technology* 37:1882-1891.
- Soller, J.A., Olivieri, A.W., Eisenberg, J.N.S., Sakaji, R., and Danielson, R. (2004) Evaluation of Microbial Risk Assessment Techniques and Applications. Water Environment Research Foundation 00-PUM-3.
- Soller, J.A., Eisenberg, J., DeGeorge, J., Cooper, R., Tchobanoglous, G., and Olivieri, A. (2006). A public health evaluation of recreational water impairment. *Journal of Water and Health* 4:1-19.
- Soller, J.A., Seto, E.Y., and Olivieri, A.W. (2007) Application of Microbial Risk Assessment Techniques to Estimate Risk Due to Exposure to Reclaimed Waters. WaterReuse Foundation, Final Project Report WRF-04-011.
- Soller, J.A., and Eisenberg, J.N.S. (2008) An evaluation of parsimony for microbial risk assessment models. *Environmetrics* 19:61-78.
- Soller, J.A. (2008) Potential implications of person-to-person transmission of viral infection to US EPA's Groundwater Rule. *Journal of Water and Health* In Press.
- Soorapanth, S., Chick, S.E., and Koopman, J.S. (2001) Simulation of stochastic infection transmission models that inform water treatment policy decisions. Pp. 517-521 in: Proc. of the 13th European Simulation Symposium: Simulation in Industry, Marseille, France, Giambiasi, N., and Frydman, C. (eds). SCS International Publication.
- Stone, P. (2006). *EU Private International Law: Harmonization of Laws* (Elgar European Law Series). Cheltenham UK: Edwin Elgar Publishing Limited.
- Teunis, P.F., van der Heijden, O.G., van der Giessen, J.W.B., and Havelaar, A.H. (1996) The Dose-Response Relation in Human Volunteers for Gastro-intestinal Pathogens. RIVM (National Institute of Public Health and the Environment) Report No. 284550002.
- Teunis, P.F.M., Medema, G.J., Kruidenier, L., and Havelaar, A.H. (1997) Assessment of the risk of infection by *Cryptosporidium* or *Giardia* in drinking water from a surface water source. *Water Research* 31:1333-1346.

- Teunis, P.F.M., and Havelaar, A.H. (1999) *Cryptosporidium* in Drinking Water: Evaluation of the ILSI/RSI Quantitative Risk Assessment Framework. RVIM Report No. 284 550 006. Bilthoven, The Netherlands.
- Teunis, P.F.M., and Havelaar, A. (2000) The beta-Poisson model is not a single hit model. *Risk Analysis* 20(4):513-520.
- Teunis, P.F., Chappell, C.L., and Okhuysen, P.C. (2002) *Cryptosporidium* dose response studies: Variation between isolates. *Risk Analysis* 22(1):175-183.
- Teunis, P., Takumi, K., and Shinagawa, K. (2004) Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analysis* 24(2):401-407.
- Teunis, P.F.M., van den Brandhof, W., Nauta, M., Wagenaar, J., van den Kerkhof, H., and Van Pelt, W. (2005) A reconsideration of the *Campylobacter* dose-response relation. *Epidemiology and Infection* 133:583-592.
- Teunis, P.F.M., Ogden, I.D., and Strachan, N.J.C. (2008a) Hierarchical dose response of *E. coli* O157:H7 from human outbreaks incorporating heterogeneity in exposure. *Epidemiology and Infection* 136(6):761-770.
- Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Pendu, J.L., and Calderon, R.L. (2008b) Norwalk virus: how infectious is it? *Journal of Medical Virology* 80(8):1468-1476.
- Thoeys, C., Van Eyck, K., Bixio, D., Weemaes, M., and De Gueldre, G. (2003) Methods Used for Health Risk Assessment in State of the Art Report: Health Risks in Aquifer Recharge Using Reclaimed Water. WHO.
http://www.who.int/water_sanitation_health/wastewater/en/wsh0308chap4.pdf
- Trachoo, N. (2003) *Campylobacter jejuni*: an emerging pathogen. *Songklanakarin Journal of Science and Technology* 25(1):141-157.
- Voysey, P.A., and Brown, M. (2000) Microbiological risk assessment: a new approach to food safety control. *International Journal of Food Microbiology* 58(3):173-179.
- Wyatt CR. (2000) *Cryptosporidium parvum* and mucosal immunity in neonatal cattle. *Anim Health Res Rev* 1(1):25-34.
- Weir, E. (2000) *Escherichia coli* O157:H7. *Canadian Medical Association Journal* 163(2):205.
- Wershil, B.K., and Furuta, G.T. (2008) 4. Gastrointestinal mucosal immunity. *Journal of Allergy and Clinical Immunology* 121(2 Suppl):S380-S383.
- Westrell, T., Bergstedt, O., Stenström, T.A., and Ashbolt, N.J. (2003) A theoretical approach to assess microbial risks due to failures in drinking water systems. *International Journal of Environmental Health Research* 13:181-197.

Westrell, T. (2004) Microbial Risk Assessment and its Implications for Risk Management in Urban Water Systems. Doctoral Thesis, Linköping University, Sweden, The Tema Institute, Department of Water and Environmental Studies.

<http://www.diva-portal.org/liu/abstract.xsql?dbid=4880>

Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, M.J., and Roderick, P.J. (1999) Study of infectious intestinal disease in England: Rates in the community, presenting to general practices, and reported to National Surveillance. *British Medical Journal* 318:1046-1050.

WHO (World Health Organization) (2001) Water Quality: Guidelines, Standards and Health: Assessment of Risk and Risk Management for Water-Related Infectious Disease. Fewtrell, L., Bartram, J. (eds.) (Published on behalf of IWA Publishing, WHO and Swedish Institute for Infectious Disease Control).

http://www.who.int/water_sanitation_health/dwq/whoiwa/en/index.html

WHO/FAO (Food and Agriculture Organization of the United Nations) (2002) Risk assessments of *Salmonella* in eggs and broiler chickens. Geneva:WHO.

Williams, T. (1965) The basic birth-death model for microbial infections. *Journal of the Royal Statistical Society, Part B* 27(2):338-360.

Wooldridge, M., and Schaffner, D., eds. (2008) Qualitative Risk Assessment. In: Microbial Risk Analysis of Foods. Washington, DC: ASM Press.

Xiao, L., Morgan, U.M., Fayer, R., Thompson, C., and Lal, A.A. (2000) *Cryptosporidium* systematics and implications for public health. *Parasitology Today* 16(7):287-292.

Zhu, J., Miller, M.B., Vance, R.E., Dziejman, M., Bassler, B.L., and Mekalanos, J.J. (2002) Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences* 99(5):3129-3134.

APPENDIX A. Flow Diagrams for Various Types of Microbial Risk Assessments

Risk assessment to characterize the risk associated with a particular pathogen/exposure combination

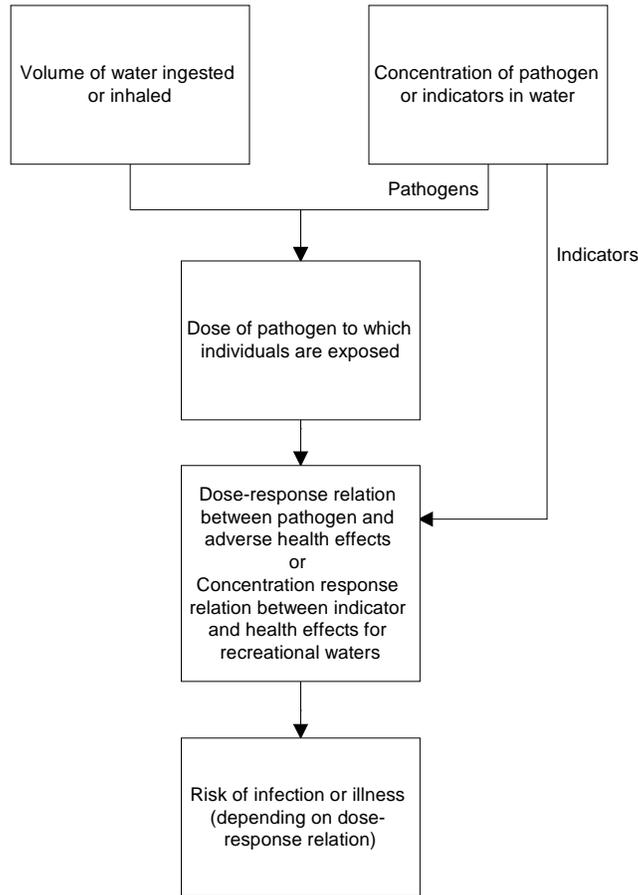
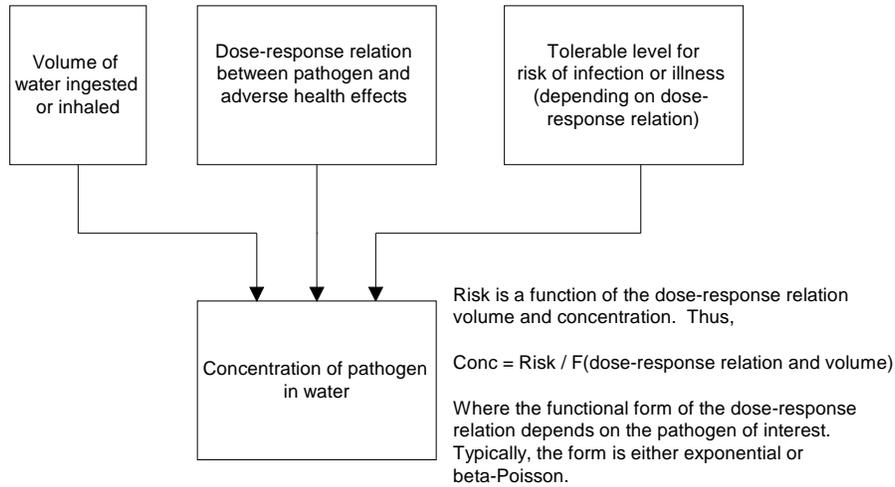


Figure A.1. Pathogen Specific Risk Assessment Flow Diagram

Risk assessment to determine ambient water concentration associated with a particular level of tolerable risk

For pathogens



For indicators in recreational waters

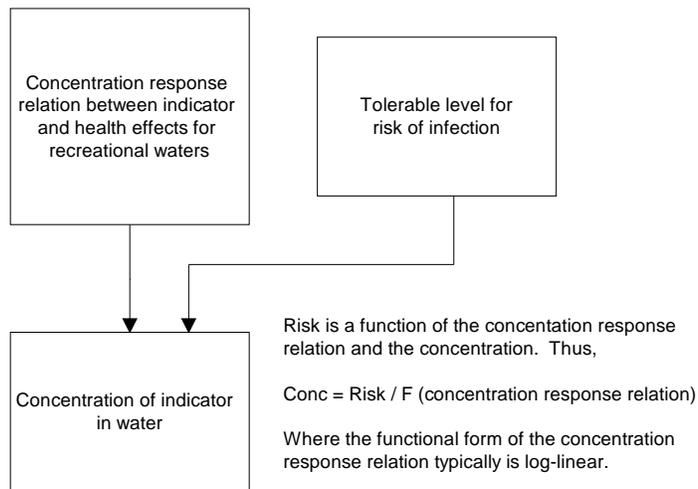


Figure A.2. Risk Assessment with Occurrence Output Flow Diagram

Risk assessment to evaluate risk ranking for a series of pathogen/exposure combinations

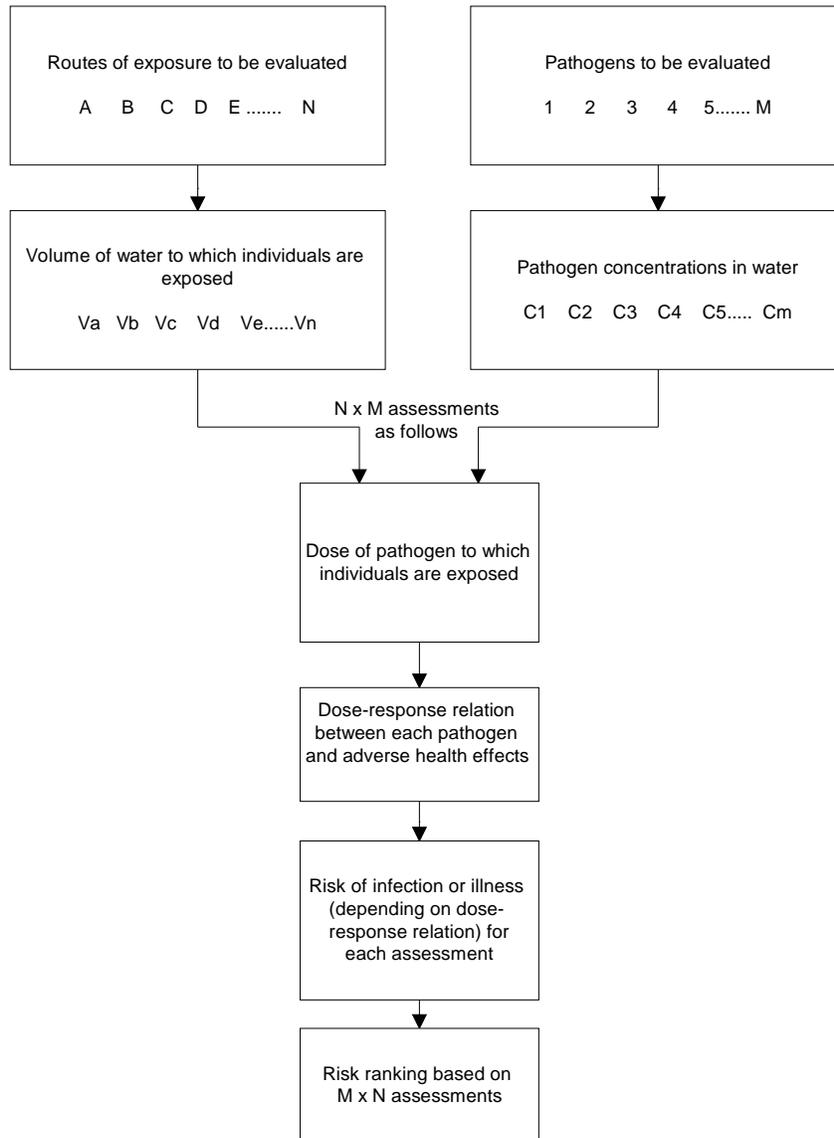


Figure A.3. Risk Assessment to Rank Pathogens Flow Diagram

APPENDIX B. Factors Unique to Microbial Risk Assessment as Compared to Chemical Risk Assessment

Chemical risk assessment methods, which were developed prior to microbial risk assessment methods, were examined for their applicability to microbial risk assessment by an EPA Office of Water workgroup. Many of the concepts developed for chemical risk assessments have parallels in microbial risk assessment, but additional features have been developed for microbial risk assessment to account for the differences between chemicals and microbes. Microbial risk assessments from the early 1990s identified several areas where chemicals and microorganisms differ, including the following:

Microbial Growth and Death

Pathogens can multiply in the environment and in a host, and are variably impacted by environmental and treatment factors. Different species, and even different strains within a pathogenic 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. Not all methods used to detect and quantify microbes can distinguish between living and dead organisms; therefore, the assay method could impact data analysis when combining or comparing studies. A further complication is that several species of bacteria, including frank pathogens (e.g., *Vibrio* spp.), have been found to exist in a state called “viable but non-culturable” or VBNC. This means that, although unable to multiply on agar-medium culture plates or grow in liquid media, such cells remain functional and metabolically active (NRC, 2004). Whether pathogens in the VBNC state are infectious has not been conclusively determined (Bogosian and Bourneuf, 2001). It is not known whether many pathogens and indicator bacteria express this trait, or whether bacteria in the VBNC state will confound or undermine the reliability of culture-based bacterial detection methods. In contrast, chemical quantification methods are generally more reproducible and able to reflect the “active” concentration of toxic agents.

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 in 2L of water) are not sufficiently developed to be reliable. Although fecal indicator bacteria are useful for detecting fecal contamination, indicator bacteria do not necessarily correlate with the presence of human pathogens or public health risk (NRC, 2004). Microbes are subject to environmental matrix effects that can cause uneven distribution that can result in consecutive measurements that differ significantly. Matrix effects can also affect the precision and accuracy of the analytical methods used to detect and quantify microbes in water. As noted above, microorganisms in a VBNC state are also a concern for interpretation of enumeration methods. For these reasons, enumeration methods for microbes introduce a

sufficiently high level of uncertainty that the details of those methods need to be discussed in the context of their impact on the risk assessment.

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. Strains of the same species (e.g., *Cryptosporidium parvum*) can have multiple genotypes, potentially with different virulences for human hosts (Morgan et al., 1999; Xiao et al., 2000). Some pathogens (e.g., *Helicobacter pylori*, many viruses) behave like quasi-species, which are fluctuating populations of genetically distinct variants that co-exist within a single host (Boerlijst et al., 1996; Covacci and Rappuoli, 1998). Microbes thus represent a “moving target” because the distribution of strains and virulence factors can fluctuate rapidly in a given water body (Loewe et al., 2003; NRC, 2004). Variation found in the environment can also depend on different sources and types of microbial pollution.

Host Immunity and Susceptibility

Human hosts have different susceptibilities to infection by particular pathogens, and levels of immunity against different pathogen species and strains may differ widely (i.e., variability among humans and variability among pathogens). 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. 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., HIV), and a number of other factors (e.g., life stages, gender, genetics).

Dose-Response Range can be Broad

The levels of pathogens required to cause infection and/or disease can vary substantially across pathogen species. Even within a particular species, those levels can vary by orders of magnitude, depending on the strain. The possible host responses may encompass asymptomatic infection, symptomatic infection (illness or disease, including chronic sequelae), and even death. Quantitative data on the exposed population’s immunity and susceptibility to a pathogen and data on pathogen strain infectivity in human subgroups with differing immunity would allow the development of dose-response curves that represent a range of possible dose-response relationships. However, these types of data are not readily available. For example, although human dose-response data for six isolates of *Cryptosporidium* are available, the data only include responses from healthy adult volunteers (for ethical reasons).

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.³³ For example, in one

³³ Chemicals that are on exposed individuals’ clothing or skin can be transferred to household and other contacts.

investigation that studied person-to-person transmission of infection, the effect of rotavirus transmission within households and on the risk of infection from outside of the household was investigated through analyses of serum pairs (Koopman et al., 1989).³⁴ In that study, infection was determined using the Wa human rotavirus antibody with an enzyme-linked immunosorbent assay. Serologic observations on 1508 individuals from 1977 through 1981 were analyzed. These data indicate that 17 to 20% of rotavirus infections were acquired in the household and the remainder acquired in the community. Based on those results it can be inferred that person-to-person transmission of rotavirus infection was responsible for at least 17% of the rotavirus transmission in the community.

Heterogeneous Spatial and Temporal Distribution

Pathogens are typically heterogeneous in environmental matrices. Whereas most soluble chemicals diffuse evenly in water matrices, pathogens may clump or may be embedded in or attached to organic and inorganic particulate debris, making concentration determinations difficult. Although concentration in pipe scale and biofilms is also a problem for chemical contaminants, some pathogens can grow and/or be protected in these environments. Also, many types of pathogens occur only episodically in drinking and source waters (and in ambient waters as well) and typically can be found only during short-lived disease outbreaks (i.e., epidemics) in a community. Seasonal increases in the environment cause water or wastewater to be contaminated episodically, through breakdowns in wastewater management or water contamination controls. Therefore, contamination sources may be different for each contamination event. Seasonal fluctuations are thought to occur due to fluctuations in factors such as precipitation, temperature, nutrient availability, human activity, and livestock events (e.g., birthing season) (NRC, 2004). The episodic nature of contamination makes calculation of relative sources of microbial contamination less useful than relative source contribution for chemicals.

³⁴ Serum antibodies, which are specific to different pathogen strains, indicate an immune response in an individual and are interpreted as an indicator of exposure to the specific pathogen strain for which antibodies are present.

APPENDIX C. Other Risk Frameworks That are Consistent with the MRA Protocol Framework

- The WHO *State of the Art Report: Health Risks in Aquifer Recharge Using Reclaimed Water* has a chapter on methods for health risk assessment that includes a process diagram for risk assessment (Thoeve et al., 2003).
- Quantitative microbial risk assessment (QMRA) approaches, along with summaries of six research papers related to health risks from infectious microorganisms transmitted via urban water and wastewater systems, are presented in this dissertation (Westrell, 2004). Discussions of susceptibility and immunity, sensitive subpopulations, secondary transmission, dynamic modeling, and health indices are also included.
- Rose and Grimes (2001) present a flow diagram for conducting a screening level risk assessment (preliminary risk assessment) that advances users through nine questions to ask during the planning of a screening level risk assessment. Molecular tools for characterizing and identifying microorganisms are also reviewed.
- Medema and Smeets (2004) discuss the interaction between QMRA and the risk management aspects of the WHO Water Safety Plan.
- The Canadian report, *Microbial Risk Assessment as a Foundation for Informed Decision-Making* (Fazil et al., 2005), presents MRA in its larger context by discussing enabling legislation, policy scrutiny, and international trade agreements and standards. The “current status” as well as “the way ahead” is presented for prioritization and coordination; methods and tool development; guidance documents (qualitative, technical, and methodology); training for risk assessors; and risk-based decision making, peer review, and integration of risk communication.
- The aim of the MICRORISK Project (www.microrisk.com) is to develop a MRA process that contributes to the decision making process for risk management of drinking water. The elements of the framework are the Quantitative Microbial Risk Assessment (QMRA) and Hazard Analysis and Critical Control Points (HACCP). Funding entities include the following: collaborative water utilities in the Netherlands (BTO), U.K. Water Industry Research, and the Australian Commonwealth Government Department of Education Science and Technology.

APPENDIX D. MRA General Concepts

Many of the microbial risk assessments conducted in the 1990s and early 2000s spurred the development of methodological tools and provided lessons learned that advanced the field of MRA. The differences between chemical and microbial risk assessment was one of the first topics the field of MRA needed to clarify and address, so that MRA could not only build on the strengths of chemical risk assessment, but also diverge where important differences were identified. The rapid growth of the field of QMRA during the late 1990s and early 2000s—particularly for water and food safety—helped to identify important differences between chemical and microbial risk assessment and facilitated the development of MRA frameworks. Prior to 2006, risk assessments for the following microbes in food or water were conducted by FDA, USDA, EPA, and the Food and Agriculture Organization with the World Health Organization (FAO/WHO), and published by their sponsoring organizations.³⁵

- *Campylobacter jejuni*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Cryptosporidium parvum*
- *Enterococcus faecium*
- *Enterobacter sakazakii*
- *Escherichia coli* O157:H7
- *Listeria monocytogenes*
- *Salmonella enteritidis*
- *Vibrio cholerae*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*.

These government-conducted risk assessments listed above were all large, multi-disciplinary team efforts that required years to develop. Each will likely be revisited and revised as new data become available and new risk management approaches are evaluated. Other countries and academic researchers have published numerous MRAs covering various environmental media and pathogens. However, the lessons learned during the U.S. Federal government-conducted risk assessments have led to increased knowledge about how to address important aspects of MRA, such as involving stakeholders, and ensuring data quality, transparency, and adequate peer review. Experience indicates that proper integration and application of the concepts discussed in this introduction should produce risk assessments that are more readily accepted by the U.S. public because the process used to plan and conduct the risk assessment and communicate findings to the public strongly influences the degree of public acceptance that can be achieved.³⁶

³⁵ Source: <http://www.foodrisk.org>

³⁶ <http://www.foodrisk.org/RACMRAFexecsummary.html>

D.1 Iterative Nature of Risk Assessment

During any of the three phases of the MRA—problem formulation, analysis, and risk characterization—other phases might be revisited and refined. For example, if during analysis new data are identified, the problem formulation might be revisited to include an input for these new data in the conceptual model. Likewise, during risk characterization, the final risk estimate might seem implausible when compared to data from well-characterized exposed populations. In this case, the analysis phase might be reviewed for errors or to incorporate better information. The iterative nature of risk assessment is indicated by the double arrows in Figure 3 (Chapter 1). All the frameworks and guidelines consulted for this MRA Protocol included the concept that risk assessment is iterative. To document the iterative nature of the process, a chronology of technical and scientific reviews can be included in the final risk assessment documentation.

D.2 Transparency, Clarity, Consistency, and Reasonableness (TCCR)

The EPA Science Policy Council *Risk Characterization Handbook* (EPA, 2000b) contains a discussion of transparency, clarity, consistency, and reasonableness (TCCR) criteria that will allow independent evaluation and interpretation of risk assessment results. A summary table of the TCCR principles is reproduced below (see Table D.1). *Transparency* is the principle value from among the four TCCR values because, when followed, it should lead to clarity and allows the reader to assess consistency and reasonableness. For risk assessments to be transparent, methods and assumptions should be clearly stated and understandable to the intended audience, whether this consists of informed analysts in the field, risk managers, or the general public. The intended audience should be able to evaluate the adequacy of the data and methods from the provided information (ILSI, 2000). Transparency also means that conclusions drawn from research are identified separately from policy judgments and risk management decisions, and that the use of default values or methods, as well as the use of assumptions in risk assessments, are clearly articulated. *Clarity* refers to the manner in which the risk assessment is presented, such as writing style and the use of graphic aids. *Consistency* provides a context for the reader, such as whether the conclusions are in harmony with relevant Agency policy, procedural guidance, and scientific rationales, and if not, how and why the conclusions differ. The *reasonableness* criteria address the extent to which professional judgments and assumptions are well founded, as confirmed by expert peer review. Risk characterizations should be consistent in general format, but recognize the unique characteristics of each specific situation.

D.3 Data Quality

Methods for evaluating data quality and performing peer review are important tools for producing a risk assessment that has both scientific value and credibility with stakeholders. The collection, use, and dissemination of information of known and appropriate quality underlie all environmental management and health protection decisions. Reasonable and timely availability and access to the information and analytical tools necessary to make and understand such decisions are essential for assessing environmental and human health risks, designing appropriate policies and response strategies, and measuring environmental improvements.

The TCCR principles contain elements also included in data quality and peer review.

Table D.1. Summary of TCCR Principles (Source: EPA, 2000b)

| Principle | Definition | Criteria for Good Risk Characterization |
|------------------|--|---|
| Transparency | Explicitness in the risk assessment process | <ul style="list-style-type: none"> • Describe assessment approach, assumptions, extrapolations, and use of models • Describe plausible alternative assumptions • Identify data gaps • Distinguish science from policy • Describe uncertainty • Describe relative strength of assessment |
| Clarity | The assessment itself is free from obscure language and is easy to understand | <ul style="list-style-type: none"> • Employ brevity • Use plain English • Avoid technical terms • Use tables, graphics, and equations |
| Consistency | The conclusions of the risk assessment are characterized in harmony with other EPA actions | <ul style="list-style-type: none"> • Follow statutes • Follow Agency guidance • Use Agency information systems • Place assessment in context with similar risks • Define level of effort • Use review by peers |
| Reasonableness | The risk assessment is based on sound judgment | <ul style="list-style-type: none"> • Use review by peers • Use best available scientific information • Use good judgment • Use plausible alternatives |

Section 515 of the Treasury and General Government Appropriations Act for Fiscal Year 2001 (Public Law 106-554, also known as the “Data Quality Act” or “Information Quality Act”) directs the Office of Management and Budget (OMB) to issue government-wide guidelines that “provide policy and procedural guidance to Federal agencies for ensuring and maximizing the quality, objectivity, utility, and integrity of information (including statistical information) disseminated by Federal agencies.” In compliance with OMB, EPA published *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of Information Disseminated by the Environmental Protection Agency* (EPA, 2002a).

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. They create a mechanism that enables the public to seek and obtain, as appropriate, correction of information disseminated by the EPA that does not comply with the EPA or broader OMB Information Quality Guidelines. However, where EPA has provided a structured opportunity for public comment on information in a draft or proposed document, EPA generally treats requests for correction procedurally like other public comments, addressing them in the formal response to comments rather than through a separate response process. In this regard, EPA believes that the public comment process serves the purposes of the Information Quality Guidelines and provides a reasonable, non-duplicative opportunity for correction of any information that does not comply with the Guidelines. Thus, EPA will not generally consider a request for correction that could have been submitted during the comment period of a rulemaking or other action.

D.4 Data Evaluation

There are general criteria for evaluating data to decide if it should be included in a risk assessment. Basic questions to evaluate data include the following (adapted from EPA, 1998):

1. Are the study's objectives relevant to the risk assessment? The most relevant data for risk assessment are those that focus on the (1) organism of interest; (2) population at risk; and (3) circumstances of exposure (e.g., vehicle, level, timescale, route).
2. Are the variables and conditions the study represents comparable with those important for the risk assessment?
3. Is the study design adequate to meet its objectives?
4. Was the study conducted properly?
5. Were there associations between observable data and the outcomes (health or otherwise) of interest?
6. Are factors that could increase or attenuate risk (risk factors) controlled for in the data?
7. How are variability and uncertainty treated in the study report?
8. Are the data sufficiently robust to be used to support a causal effect between exposure and infection or illness?

D.5 Data Uncertainty and Variability

For risk assessment, it is important to understand the difference between uncertainty and variability, how they can be related, and how they impact the quality of model predictions. The EPA *Exposure Factors Handbook* advises the following (EPA, 1997a, 2000b):

While some authors have treated variability as a specific type or component of uncertainty, the EPA has advised the risk assessor (and, by analogy, the exposure assessor) to distinguish between variability and uncertainty. Uncertainty represents a lack of knowledge about factors affecting exposure or risk, whereas variability arises from true heterogeneity across people, places, or time. In other words, uncertainty can lead to inaccurate or biased estimates, whereas variability can affect the precision of the estimates and the degree to which they can be generalized.

Part of the confusion arises from a failure to distinguish between the characteristics of model *input* data and assumptions and characteristics of model *outputs*. When evaluating model inputs, the classical distinction between uncertainty and variability is usually clear, at least in principle. When evaluating model outputs, the term "uncertainty" usually refers to the overall precision of risk or parameter estimates, and this uncertainty, or lack of precision, arises from a combination of uncertainty and variability in the input data and model specification. Uncertainty and variability should be considered separately in risk assessment (Nauta et al., 2009). The following sections address variability in uncertainty in model inputs, unless otherwise noted.

Variability

To the extent allowed by available resources, variability can be increasingly characterized by gathering additional data. For example, the levels of *Cryptosporidium* in a source water would be better characterized by obtaining oocyst measurements every day over the course of a year,

rather than quarterly. Increased sampling frequency would reveal how the levels of *Cryptosporidium* fluctuate as seasons change and precipitation events occur. However, if several inputs to a risk assessment are highly variable it may not be possible to gather sufficient data to characterize exhaustively the relationships between input variability and model predictions. The important point in any assessment is to understand how specific limitations in data may affect the ability to make reliable model predictions. Also, no amount of data can reduce the magnitude of variability in any parameter value or change the effect of variability on model outputs; additional data can only more accurately define the existing variability.

Data variability may be represented by confidence intervals around a point estimate, percentiles, or through more complex statistical distributions. Three types of variability common to microbial risk assessment include the spatial and temporal variability in exposure levels and inter-individual variability in behaviors related to exposure and in susceptibility to infection. There are several ways variability can be addressed in risk assessment (adapted from EPA, 1995a; Morgan and Henrion, 1990):

- Use point estimates; do not quantify uncertainty/variability—this is only appropriate in the rare cases when all the sources of variability are clearly known to have small effects on model results (e.g., default adult body weight).
- Disaggregate variability (stratify)—variability is smaller within each group (e.g., develop distributions of body weights based on age and gender).
- Use central tendency (average or median) value—ignores “tails” of distribution such as highly exposed or most sensitive individuals.
- Use upper or lower-bound estimates—useful as a screening level approach, but can lead to unrealistically high or low risk estimates. For example, if multiple conservative parameter value estimates are combined in a single model an unrealistically high risk estimate may result (see discussion of sensitivity analyses below).
- Sample from a parameter distribution—apply Monte Carlo or bootstrap approaches to derive probabilistic estimates of parameter values or microbial risks.

Uncertainty

Although variability cannot be reduced by gathering more data, some types of uncertainty can be reduced by obtaining additional information. For example, confidence intervals for a central tendency estimate, such as the mean value, become smaller if more samples or subjects are studied from the same population. In every microbial risk assessment, many model inputs have significant levels of uncertainty. By its very nature, uncertainty is hard to quantify. Very often, it is only possible to qualitatively rate the level of uncertainty as high, medium, or low; compare it with others sources of uncertainty; or to bound absolute uncertainty within order-of-magnitude limits based on physical or biological plausibility.

The 1997 EPA *Exposure Factors Handbook* identifies three general types of uncertainty in chemical risk assessment, identifies their sources, and provides examples (see Table D.2). Essentially all of these (with suitable modifications) are relevant to microbial risk assessment. Morgan and Henrion (1990) provide a related taxonomy of seven types of uncertainty in the broader area of risk and risk-related policy analysis. These include statistical variation,

Table D.2. Uncertainty and Associated Sources and Examples (Source: EPA, 1997a)

| Type of Uncertainty | Sources | Examples |
|---|---------------------|---|
| Scenario uncertainty (e.g., lack of knowledge about exposure circumstances or virulence) | Descriptive errors | Incorrect or insufficient information |
| | Aggregation errors | Spatial or temporal approximations |
| | Judgment errors | Selection of an incorrect model |
| | Incomplete analysis | Overlooking an important pathway |
| Parameter uncertainty (e.g., lack of knowledge about numerical values) | Measurement errors | Imprecise or biased measurements |
| | Sampling errors | Small or unrepresentative samples |
| | Variability | In time, space, or activities |
| | Surrogate data | Structurally-related chemicals |
| Model uncertainty (e.g., lack of knowledge about the form of important risk relationships or correlations) | Relationship errors | Incorrect inference on the basis for correlations |
| | Modeling errors | Excluding relevant variables |

subjective judgment, linguistic imprecision, variability, inherent randomness, disagreement, and approximation.

Uncertainty analyses for MRA generally have the following interrelated goals:

- Identification of all important sources of uncertainty in model outputs.
- Characterization of the importance of specific sources of variability and uncertainty (from Morgan and Henrion, 1990), including the following:
 - computing the effect of changes in inputs on model predictions (sensitivity analysis);
 - calculating the uncertainty in model outputs induced by uncertainty in the inputs (uncertainty propagation); and
 - comparing the importance of uncertainties in terms of their relative contributions to uncertainty in the outputs (uncertainty analysis).
- Characterization of overall uncertainty in the risk assessment outputs (risk characterization).

It is also important to include an evaluation of potential random and systematic error and an estimate of its variability. Examples of sources of variability and uncertainty include the following:

- errors³⁷ introduced as a result of study design;
- errors associated with estimates of the concentration of the pathogenic microorganism;
- errors associated with estimates of human ingestion volumes;
- relevant data may not be available for all aspects of the analysis;
- data may be of questionable quality;
- data limitations and incomplete knowledge of the underlying biological mechanisms of growth and disease can lead to uncertainty in MRAs; and
- variability due to the range of the genetic composition of hosts and pathogens, to various environmental conditions, and to different health conditions of individuals.

³⁷ Error in this case refers to statistical error bars, not errors in logic.

Microbial risk assessments are often subject to uncertainty about the (1) knowledge available about the pathogens, host characteristics, and health outcomes; (2) models used to characterize the relationship between exposure and health outcome; and (3) available quantitative information for various parameters.

Documenting Uncertainty

Discussion of sources of uncertainty should appear throughout the risk assessment documentation, as well as be compiled in a summary of assumptions and their uncertainties. Methods for addressing uncertainty can and should be integrated into the risk assessment to the extent justified by the quality of the data and required by the risk management options being considered. However, this approach can decrease transparency if the method is not clearly described. Clear documentation of the method for addressing the uncertainty and the justification for the method should be included in the risk assessment. This is one of the most important areas for transparency because a lack of transparency can cause multiple methods to account for the same uncertainty to be layered and result in an inaccurate picture of risk.

In every risk assessment, it is important for risk assessors to communicate to risk managers the types and magnitudes of uncertainties in the risk assessment. This information should also be presented in a way that is transparent for peer reviewers and stakeholders. Sometimes, risk managers apply an adjustment to the risk assessment output to account for all the uncertainties (e.g., using an upper confidence limit from the risk model output). In such situations, the risk assessment is not altered, but the justification of the adjustment should be stated by risk managers.

Unlike chemical risk assessment, uncertainty factors (UF), safety factors (SF), modifying factors (MF), and explicit margins of safety (MOS) have not been used in microbial risk assessment. These methods for characterizing uncertainty are summarized below for completeness; however, it should be clear that their inclusion in this Appendix does not condone their use in MRA. In the future, EPA will continue to address uncertainty in MRA on a case-by-case basis where the available data should be applied as appropriate to ensure health-protective and reasonable MRAs are conducted.

Uncertainty Factors (UF): The use of UFs is one method that is commonly used in chemical risk assessments to account for existing data gaps with the goal of arriving at a risk estimate that is more protective of human health than if the UF had not been applied. Uncertainty factors are numerical values (often a factor of 3, 5 or 10) applied to a toxicological reference value to account for the fact that the reference value might not represent the endpoint of concern in humans. Although EPA provides general guidance for choosing UFs in chemical risk assessments, professional judgment is important in UF selection. Uncertainty factors are applied by dividing the value to which the UF is being applied by the UF (e.g., an UF of 10 applied to a value of 36, results in a new value of 3.6). The use of UFs as currently defined for other fields of risk assessment, such as chemical risk assessment, have no precedent in the field of MRA. To date, none of the MRAs conducted by FAO/WHO, FDA, USDA, or EPA have used UFs.

Safety Factors (SF): Safety factor is a term used by FDA that is similar to UF as summarized

above. The application of a safety factor is required by law for food additives. For example, 21 CFR 70.40 states the following:

The following safety factor will be applied in determining whether the proposed use of a color additive will be safe: Except where evidence is submitted which justifies use of a different safety factor, a safety factor of 100 to 1 will be used in applying animal experimentation data to man; that is, a color additive for use by man will not be granted a tolerance that will exceed 1/100th of the maximum no-effect level for the most susceptible experimental animals tested.

A SF of 100 indicates that a concentration is divided by 100 to arrive at a more conservative concentration. The use of explicit SFs as defined above, have no precedent in the field of MRA.

Margin of Safety (MOS): A margin of safety can also be used to address uncertainty. For example, the CWA requires that an MOS be included in the calculation of TMDLs for determining wasteload allocations in support of meeting water quality standards (EPA, 2001). Two types of MOS can be applied for TMDLs, (1) an implicit MOS is accounted for through conservative assumptions in the analysis (to justify this type of margin of safety, an explanation of the conservative assumptions used is needed); and (2) an explicit MOS is incorporated by setting aside a portion of the TMDL as the MOS.

The Food Quality Protection Act of 1996 states that for threshold effects (from chemical exposures), “an additional tenfold margin of safety for the chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of data with respect to exposure and toxicity to infants and children.”

Implicit MOSs are often used in the form of conservative assumptions during screening level MRAs. Within this context their use is justified.

Adjustment Factors (AF): One method that has been used in MRA to account for uncertainty is the application of an adjustment factor, which are generally applied where data can direct the magnitude and the direction of adjustment of a value. Adjustment factors are different than UFs, MFs, SFs, and an MOS, because data on specific organisms and exposure scenarios are used to estimate the AF. There are no default adjustment factors because adjustment factors are derived from that are applicable to the specific scenario or similar scenarios. An explanation of the data used to derive the AF and how those data are relevant to the scenario should be included. Some examples of adjustment factors for addressing parameter uncertainty are provided in Text Box D.1.

D.6 Model Validation

Model validation and verification in risk assessment are general terms that are sometimes used to refer to rigorous data driven evaluation of models, but more often they are used interchangeably to refer to a less rigorous “reality check” that may have poorly defined validation criteria. Risk assessors should be aware of the differences between model validation and verification and whether a model has been validated for interpolation or extrapolation. The USDA ARS has

Text Box D.1. Examples of Addressing Parameter Uncertainty

Mortality Adjustment Factor—for drinking water, the LT2 risk assessment used the following mortality rates: unfiltered systems of 16.63 deaths per 100,000 cryptosporidiosis illnesses (14.65 AIDS + 1.98 non-AIDS); and for filtered systems 10.55 deaths per 100,000 cryptosporidiosis illnesses (8.57 AIDS + 1.98 non-AIDS). The mortality factors were constants in the model (i.e., no uncertainty was attributed to these parameters). The mortality factors were arrived at by using the Milwaukee AIDS-related mortality rate with AFs to account for differences between 1993 and 1999 in both the general AIDS mortality rate and the difference in percentage of the national population that was living with AIDS (EPA, 2003b). This factor adjusts for the increased susceptibility of AIDS patients to death from cryptosporidiosis.

Dose-Response Adjustment Factor—an example of where an AF was used to shift a dose-response curve in the opposite direction that an UF would shift a curve is provided by the FDA/USDA *Listeria monocytogenes* risk assessment (FDA/USDA, 2003):

The dose-response curve derived from the mouse study estimates that the LD₅₀ is about 4.26 logs or 20,000 cfu. The exposure data indicate that exposure to this number of *L. monocytogenes* is relatively frequent. If the mouse dose-response model were directly applicable to humans, the dose-response model would overestimate the number of human deaths due to listeriosis by a factor of over one million. This indicates that normal human beings are much less susceptible to *L. monocytogenes* than laboratory mice... A dose-response adjustment factor was developed to correct the mouse-derived model so that it was applicable to humans. The size of this factor is determined by surveillance data reported to FoodNet for each of the subpopulations modeled in this risk assessment... While the shape of the dose-response curve is initially derived from mice, the scale is determined by the human epidemiology.

ongoing research in predictive microbial growth that is used to create their PMP.³⁸ Researchers gathering data for that Program use the more formal rigorous definitions of verification and validation as described below (Oscar, 2005):

The use of the terms verification and validation is controversial because in predictive microbiology, these terms are used as synonyms, whereas in other fields of science they are not. More specifically, verification is the successful outcome of the performance evaluation process where the model predictions were compared with the data used in model development (that is, dependent data). In contrast, validation is the successful outcome of the performance

evaluation process where model predictions were compared with data that was not used in model development (that is, independent data). Although use of the terms verification and validation may be at odds with their current usage in predictive microbiology, separate usage of these terms has the advantage of providing an easy and needed distinction between the 2 types of evaluation processes, that is, 1 with dependent data and 1 with independent data. Furthermore, the use of the terms is consistent with their usage in other scientific disciplines.

In the context of pathogen growth models based on laboratory studies of growth in broth, Oscar (2005) indicates that “proper evaluation of model performance for extrapolation requires an independent set of data that differs from the data used in model development by only 1 variable.

³⁸ Version 7.0 <http://www.arserrc.gov/mfs/Download.htm>

However, use of datasets that differ by more than 1 variable from the dataset used in model development will confound the comparison of observed and predicted values and thus invalidate the performance evaluation for extrapolation.” It should be noted that other types of models used in risk assessment, such as dose-response models, are not validated in the same way as the pathogen growth models (see more below).

Because validation implies different criteria in different situations, any discussion of validation should refer to how the validation was performed so that readers may understand the degree of rigor the validation effort entailed. One method of validating the risk assessment findings is to compare the outputs to epidemiological data to determine whether the risk estimates are consistent with reality. Text Boxes D.2 and D.3 provide examples of MRA model validation. The FAO/WHO *Hazard Characterization for Pathogens in Food and Water Guidelines* (FAO/WHO, 2003) includes discussion of validation of dose-response models, including conceptual validation, validation of algorithms, validation of software code, and functional validation.

Text Box D.2. Rotavirus in Drinking Water

To confirm the validity of the output results of the epidemiologically-based model used in a case study of rotavirus in drinking water (Soller et al., 1999), a dynamic model was modified using actual data and best judgment to analyze and simulate a 1981 rotavirus outbreak in the Eagle-Vail and Avon communities in Colorado (Hopkins et al., 1984). Although a rigorous direct comparison of the results from the actual outbreak and the rotavirus simulation could not be conducted due to a lack of specific surveillance data (e.g., concentration data, secondary spread), a qualitative comparison was made to assess the plausibility of the output from the model. The overall attack rate for diarrhea and/or vomiting during the rotavirus epidemic was reported to be approximately 32% (Hopkins et al., 1984). Using virus detection or serological methods, it was estimated that a total of approximately 23% of the population became ill from rotavirus exposure during this event. The results of a 5000 trial Monte Carlo simulation of the outbreak using the model showed that about half of the trials resulted in average daily disease prevalence rates ranging from 7.5% and 25%, which compares favorably to the historical estimate of 23%. Thus, it may be inferred that the output from the model seems plausible and intuitively consistent with the actual outbreak data.

Text Box D.3. *Cryptosporidium* in Drinking Water

Teunis and Havelaar (1999) conducted a case study of *Cryptosporidium* in drinking water and discussed the importance of and opportunities to attempt validation of their calculated estimates of yearly individual infection risk through comparison with actual epidemiological data on endemic/epidemic cryptosporidiosis. Their approach also provides a logical and transparent methodology to integrate quality of life-based approaches into the risk assessment by expressing all health effects in one single metric—the DALY. Such an approach has the added advantage of not being disease-specific and lends itself for risk comparisons (e.g., with chemical risks, for economic evaluations).

D.7 Expert Opinion

Experts are involved in all aspects of risk assessment; however, this section addresses the specific use of experts regarding quantitative estimates of risk. Sometimes important parameters in the risk assessment do not have values that are well supported by available data, or the relationship between parameters is suspected, but not supported by robust data. In these situations, expert opinion may be used to fill in the data gaps or address uncertainty (such as model uncertainty). To help readers judge the quality of the expert opinion, the method used to elicit the expert opinion should be described. This MRA Protocol, however, does not provide a detailed discussion of expert opinion elicitation methods. Readers are referred to Ouchi (2004) and Morgan and Henrion (1990) for summaries of methods and citations for primary literature in the field of expert elicitation. Some of the methods summarized by Ouchi (2004) and Morgan and Henrion (1990) include the following:

- Behavioral approaches
 - Face-to-face interaction
 - Delphi method
 - Nominal group technique
- Mathematical approaches (for probabilistic risk analysis)
 - Non-Bayesian axiomatic models (opinion pools, performance-based weight model)
 - Bayesian models (additive error and multiplicative error models, stochastic dependence)
 - Psychological scaling models (Thurstone model, Bradley-Terry model, negative exponential lifetime model).

It should be noted that Morgan and Henrion (1990) observed that “because the public decision maker must often informally factor in a number of other considerations, it is rarely of great practical consequence that a more formal treatment in the combining or weighting of alternative expert views is not possible.”

A recent example of how EPA has used expert judgment is the Office of Air Quality Planning and Standards (OAQPS) report *An Expert Judgment Assessment of the Concentration-Response Relationship Between PM_{2.5} Exposure and Mortality* (EPA, 2004a). Based on an NRC report recommendation, OAQPS used expert judgment to develop probability distributions for key sources of uncertainty regarding the mortality effects of ambient fine particulate matter (PM_{2.5}) exposure. EPA’s Environmental Benefits Mapping and Analysis program (BenMAP)³⁹ includes the exposure-response functions derived through expert judgment assessment as options for risk modeling.

Criteria for identification and selection of experts are not well developed or formalized. EPA’s *Peer Review Handbook* (EPA, 2000a) provides guidance on selection of peer reviewers that includes where to find peer reviewers, what mix of expertise may be important, representing diversity of disciplines, and limiting conflicts of interest. According to the National Committee on Radiation Programs (NCRP, 1996), an expert has the following characteristics:

³⁹ <http://www.epa.gov/ttnecas1/benmodels.html>

- training and experience in the subject area resulting in superior knowledge in the field;
- access to relevant information;
- an ability to process and effectively use the information; and
- is recognized by his or her peers or those conducting the study as qualified to provide judgments about assumptions, models, and model parameters at the level of detail required.

D.8 Risk Assessment Team

The material in this section provides a summary of the discussions from EPA's problem formulation workshop (EPA, 2003c). Problem formulation (see also Chapter 2) is an iterative process that may start with a relatively small team that increases in size as expertise needs are identified and the scope is further defined. Team members' involvement may fluctuate as the scope is broadened or reduced. By the end of the problem formulation stage a working relationship of trust and respect should be established among the various categories of personnel involved. The same individuals involved in problem formulation may also be involved in overall Planning and Scoping.

Risk assessment teams are multidisciplinary and may include people with expertise in the following disciplines: economics; law; engineering; life, physical, and social science (such as microbiology, epidemiology, toxicology, chemistry, and medicine); statistics; mathematics; software programming; website design; and technical writing (such as policy documents, press releases, Federal Register notices, educational materials, and executive summaries). Individuals may have overlapping roles, but it is important that conflicts of interest between risk assessors and risk managers be avoided in order to maintain the scientific integrity of the process and stakeholder confidence. Thus, risk assessment and risk management roles for team members should be clearly defined.

It is also important to recognize that team composition may evolve throughout the problem formulation stage and that the problem may be formulated in a different way as information and additional expertise is obtained. Risk managers, risk communicators, risk assessors, and stakeholders should all be involved in the problem formulation stage of microbial risk assessment. This is important for the success of the risk assessment as measured by accomplishment of goals and acceptance of the risk assessment by interested parties. The nature and degree of involvement and interaction will vary depending on the unique aspects of the risk assessment situation. However, there are some common themes outlined in this section that can help guide the problem formulation process. The Planning and Scoping and problem formulation can form the basis of a memorandum of understanding between risk assessors and risk managers regarding how the risk assessment will be conducted. Although communication between risk assessors and risk managers take place during all phases of the risk assessment, it may be most intense during problem formulation and risk characterization.

Risk Managers

EPA has a hierarchy of risk managers, so it is appropriate for risk management activities to be

hierarchical in nature. The hierarchy of managers extends from team leaders that manage the process on a daily basis to the EPA Administrator. Different Offices have different organizational structures but the principle that decisions are made at different levels should apply to all Offices. For the purpose of this section, manager roles will be divided into three levels, program (i.e., process coordinating) managers, mid-level managers (i.e., Office directors), and senior-level Agency managers.

Planning and Scoping should outline the management hierarchy with a degree of detail that is appropriate for planning deliverables and milestones. The roles are not exclusive to each level and in practice would likely overlap considerably. Risk managers at all levels are responsible for ensuring appropriate communication with managers above and below their level. It is important to identify the team leader and his/her basic responsibilities at the outset to avoid misunderstandings as the process develops. The initial meetings should also be used to discuss the basic responsibilities of each team member, and this discussion may need to be repeated in future meetings as more members are included and as the objectives change. It should be remembered that the team will likely be composed of persons outside the immediate supervision of the risk manager (e.g., personnel in other Offices, contractors); therefore, appropriate management and interpersonal skills are required to accomplish the task.

Although a concern can be brought to the attention of an agency through many routes, most often the mid-level risk manager will be responsible for starting the Planning and Scoping process. The risk manager defines the concern and puts it in regulatory and policy context. He/she also would provide agency specific historical context regarding the issue. Lessons learned from previous agency experiences in risk assessment should be considered. Budgetary, time, and human resource constraints must also be defined at the start of problem formulation. The mid-level manager is usually responsible for assembling the initial team that will start the process. Timelines and deliverables may be adjusted as scope, goals, approach, audiences, and resources are further defined. It is important that the progress made at each risk assessment team meeting and any changes in the goals or scope are documented in written format. This facilitates rapid orientation for newcomers and allows all team members to keep track of issues that have been resolved and issues that have yet to be resolved. The mid-level and/or coordinating managers are responsible for ensuring that this is done in a timely and effective manner. The mid-level manager looks at risk assessment and timeline options, the pros and cons of various options, and decides when documents are ready for senior-level manager review. Mid-level managers may have questions and issues for various staff members (e.g., economists, scientists, lawyers) regarding different factors that might influence the scope, questions, or logistics for the planned risk assessment. Constraints that will influence the plan need to be identified. Timelines should reflect if the situation is urgent versus slowly evolving. Resources such as money, personnel, expertise, and quality and quantity of data may influence the scope or limit the breadth of questions that could be answered. Laws, regulations, and Agency policies will influence the planning and goals of a risk assessment. Social values should also be considered during the problem formulation stage. The mid-level manager is responsible for ensuring that all these factors are taken into account during the Planning and Scoping stage and may involve designing work assignments for appropriate contractors.

Because program/coordinating managers directly oversee deadlines and deliverables

development during the risk assessment, they are responsible for ensuring that logistical plans are realistic. They often act as liaisons between risk assessors and upper management and should be familiar with all aspects of the Planning and Scoping as well as all aspects of the risk assessment. They may be responsible for identifying who can best address upper management questions or concerns. Program/coordinating managers often oversee the iterative nature of problem formulation. It is the coordinating and/or mid-level manager that ensures risk assessors are following the data quality guidelines set by the Agency and that Quality Audit plans are followed. Managers at this level are also responsible for ensuring that agency peer review guidelines are followed, such as those outlined in the EPA *Peer Review Handbook* (EPA, 2000a).

Senior-level agency managers may not have frequent involvement in the risk assessment process, but should be aware of key issues and constraints. They may be able to acquire more resources or time if a clear need for additional resources is established. Visible involvement of senior managers can be important for building stakeholder trust in the process and product. Senior managers may have preferred methods for being briefed regarding project details that should be taken into account during the Planning and Scoping. Senior managers are ultimately responsible for ensuring that Agency needs and stakeholder needs are met in a balanced manner. Managers at the most senior levels will almost always be involved in risk communication activities with the public. Thus, they should be made aware of any details or issues that would assist or hinder their risk communication efforts.

All managers are responsible for ensuring that conflicting opinions of the risk assessment team or stakeholders, should they occur, are resolved with as little damage to working relationships as possible. During Planning and Scoping, managers work to define their own roles in the risk assessment process and implementation of the end product. The nature of outputs, but not the content, should be agreed upon during Planning and Scoping. For example, the nature of the output may be a risk ranking, but the actual ranking would not be known until the risk assessment is completed. Or, if the risk assessment is to determine a quantitative output, the units may be defined during problem formulation, such as the number of organisms per liter of drinking water that would result in 1 person in 10,000 becoming infected during a year of drinking that water. Planned transparent documentation of the process can greatly assist institutional memory and make future endeavors more successful.

Role of Risk Assessors

Risk assessors have more modular roles as opposed to the hierarchical roles of risk managers. They identify, inventory, and obtain data; conduct preliminary assessments; run models; and provide outputs. When the risk manager presents the case to risk assessors, the assessors evaluate the technical issues that need to be resolved to meet the risk manager's vision for the project. Risk assessors may identify technical, time, or data constraints that would need to be relayed to risk managers so that the objectives or questions can realistically be answered. Assessors and/or the coordinating manager draft and finalize the conceptual model, accompanying narrative, and analysis plan. Assessors are also responsible for the content and ensuring transparency of their work for managers and risk communicators. Assessors evaluate data quality within the context of the Agency data quality guidelines and work with managers to

make decisions about what types of data should or should not be included based on data quality and scope. Data quality requirements are different depending on the planned use of the risk assessment; however, if data are excluded, the assessment must note the exclusion and reason for the exclusion. Discussion of data quality issues should be a part of problem formulation.

Risk assessors have an important role in problem formulation because they are most familiar with the data and risk assessment approaches that may be available to conduct a quantitative risk assessment. If the data are sparse, they should inform team members and make the case for a qualitative or a less ambitious (semi-quantitative) assessment. Their input is also important in drafting the description of the problem from a more technical point of view and to note “upfront” the anticipated limitations of data and how the final product should be interpreted. Risk assessors should also advise the risk assessment team about possible revisions in the scope of work as the assessment continues, since they are the most likely to be aware of upcoming and newly published data.

Role of Risk Communicators

Risk communicators interact with risk assessment teams and all levels of risk managers. Risk communication also tends to be hierarchical. Everyone involved in the process should be communicating at least within the Agency, but one individual should have the lead for coordinating communications with outside stakeholders. The risk communicator will most likely be developing a draft risk communication strategy at the same time as the risk assessment is being planned. Communicators can often help identify team members and will frequently be familiar with stakeholders. Risk communication is seldom successful if risk communicators are brought in late or only to fix a problem. Risk communicators can help build trust with communities and stakeholders before the tough issues come up. Risk communicators and managers work together to define stakeholder roles, when they are involved.

Risk communicators are important to Planning and Scoping because they are often the ones who have experienced first hand a failure of the risk assessment process to accomplish its objective—usually due to a lack of input from communication specialists. Risk communicators can help serve as the bridge between highly technical risk assessors and less technical risk managers; they can also help ensure that stakeholders are involved in the process.

Role of Stakeholders

The term “stakeholders” usually refers to people and organizations that can shape the process or will be (or perceive themselves to be) impacted by the risk assessment. It is important that the stakeholders understand the issues, the process, and the final product that is to be produced. Thus, they should be involved in the problem formulation in some meaningful way. At a minimum, they should be informed about the problem, how it is to be addressed, and have an opportunity to provide comments. When stakeholders are directly affected by the proposed assessment, stakeholder comments should be sought to help team members better understand and define the problem. Stakeholders should also be informed periodically of any changes in the problem formulation. These actions will help avoid problems in communicating the results.

Although stakeholders may have less involvement in mandated risk assessment activities, their early involvement in voluntary situations is very important. Stakeholders include but are not limited to governmental bodies (state and Federal agencies), consumers and their organizations, representatives from industry and their organizations, trade organizations, representatives of professional organizations, representatives of educational organizations, representatives of research organizations, and the impacted public. Stakeholders can provide information on their concerns, their values, and personal data on exposures and life style (in the case of communities and workers). Stakeholders can also provide feedback on the relevance and clarity of the risk management objective, the scope for the assessment, the timing, the conceptual model, and analysis plan. They can also provide expertise in hazard and exposure assessment, technology, economic areas, social areas, political areas, and legal areas. However, it is sometimes forbidden for stakeholders to be formal members of the risk assessment team. The Planning and Scoping stage should include activities to define the stakeholders that are appropriate risk assessment partners, such as other U.S. Federal agencies.

Medema and Smeets (2004) discuss stakeholder participation from the perspective of water suppliers, regulators, consumers, inspectors, and health authorities.

D.9 Peer Review

OMB has published a *Revised Information Quality Bulletin for Peer Review* (OMB, 2004) that specifies minimum requirements for the peer review of highly influential scientific assessments. EPA's Science Policy Council's *Peer Review Handbook* (EPA, 2000a) is Agency-wide guidance on implementing EPA's peer review policy as articulated in *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (EPA, 1994). The Handbook is based, in part, on the central themes set forth in the following 1994 Policy statement:

Major scientifically and technically based work products related to Agency decisions normally should be peer reviewed...These decisions are made in conformance with program goals and priorities, resource constraints, and statutory or court-ordered deadlines. For those work products that are intended to support the most important decisions or that have special importance in their own right, external peer review is the procedure of choice. Peer review is not restricted to the penultimate version of work products; in fact, peer review at the planning stage can often be extremely beneficial.

Thus, the role of peer review is to enhance the quality and credibility of Agency decisions by ensuring that the scientific and technical work products underlying these decisions receive appropriate levels of peer review by independent scientific and technical experts. These guidelines, including flowcharts and related guidance for planning, conducting, and completing a peer review, should be consulted and followed when applicable during the MRA process.

Although stakeholders, by definition, may have conflicts of interest regarding the outcome of the risk assessment, they can provide a positive mechanism for input as well as review the risk assessment outputs. Peer reviewers, on the other hand, should have no conflicts of interest. However, if a person is sufficiently knowledgeable about an issue to be a peer reviewer, it is possible that there could be some level of conflict of interest. An example is a university researcher who recommends more investment in research that might positively impact their

field's funding situation. Peer review and stakeholder review have different goals. While some stakeholders may be able to participate in the peer review of the proposed assessment, their input is from a different perspective. EPA's Science Policy Council *Peer Review Handbook* (EPA, 2000a) should be consulted for guidance regarding EPA's peer review process.

D.10 Discussion of Risk Perception

As noted previously, risk characterization is also a key component of risk communication. Well balanced risk characterizations present information on the elements discussed above for use by other risk assessors, EPA decision makers (risk managers), stakeholders, and the public. Risk communication is an ongoing integral component of the risk assessment process. Detailed guidance for facilitating and ensuring appropriate risk communication among risk assessment team members, risk managers, and all interested parties is beyond the scope of this document. However, much of the guidance provided by this document, such as the AWQC documentation outline and the principles of TCCR, are relevant tools for risk communication.

The EPA *Science Policy Council Risk Characterization Handbook* (EPA, 2000b) recommends that risk characterization include a discussion of risk perception. A complete discussion of the risk perception issues is more appropriate for a risk communication plan; however, discussion of risk perception in the risk characterization section of the risk assessment helps clarify and reinforce the important relationship between the risk characterization output and its utility for risk communication. Any findings from studies of risk perception that relate to the hazard or a similar hazard should be presented. Questions that should be answered include the following (from EPA, 2000b):

1. What are the alternatives to this hazard? How do hazards compare?
2. How does this risk compare to other risks?
 - a. How does this risk compare to other risks in this regulatory program, or other similar risks that EPA has made decisions about?
 - b. Where appropriate, can this risk be compared with past Agency decisions, decisions by other federal or state agencies, [or international bodies] or if appropriate, to common risks with which people may be familiar?

The limitations of making comparisons should be described. Also, the significant community concerns that influence public perception of risk, if known, should be discussed.

Risk perception is influenced by so-called *outrage factors*, which are presented in Table D-3. A discussion of how the hazard is viewed by the public in the 12 areas listed below should help risk communicators to build a risk communication strategy. In addition, it may suggest which exposure scenarios will be of the most interest to the public.

Perceived increased risk to children can also contribute to public outrage.

Table D.3. Twelve Components of Outrage
 (Source: Adapted from P. Sandman)⁴⁰

| Less Risky | More Risky |
|-------------------------|-------------------------|
| Voluntary | Coerced |
| Natural | Industrial (artificial) |
| Familiar | Exotic |
| Not memorable | Memorable |
| Not dreaded | Dreaded |
| Chronic | Catastrophic |
| Knowable | Unknowable |
| Individually controlled | Controlled by others |
| Fair | Unfair |
| Morally irrelevant | Morally relevant |
| Trustworthy sources | Untrustworthy sources |
| Responsive process | Unresponsive process |

⁴⁰ <http://www.psandman.com/articles/holing.htm>

APPENDIX E. Possible Future MRA Goals and Research Needs

Some examples of possible long-term development goals for microbial risk assessment include the following:

Human Health Effects

- Developing dose-response models that consider situations where populations may be repeatedly exposed to certain microbial pathogens over time (**discrete versus continuous dose and exposure**). These models may include susceptibility and immunity variation and life stages.
- Developing **criteria for the use of animal model results** for derivation of dose-response models. Improved methods to extrapolate animal dose-response information to human dose-response models should be pursued, as well as better ways to address the uncertainty involved in such extrapolations (such as differences in health effects between humans and animals).
- Exploring the issue of whether **threshold or nonthreshold dose-response models** are most appropriate for various pathogen-host combinations.
- Developing **biologically-based mechanistic models** (such models are being developed but are not yet available).
- Develop methods to investigate **dose-response relationships for immuno-compromised and other more sensitive populations**. This could include outbreak related studies, epidemiological studies, or studies with immuno-compromised animal models.

Exposure

- Considering how **animal reservoirs of disease** might be incorporated into MRA.
- Developing better methods to account for the **heterogeneous distribution of microorganisms and the potential fluctuations in concentration** of microorganisms in the environment (spatial heterogeneity and temporal fluctuations).
- Developing methods to address **relative source contribution for microbial risks**; that is, evaluating the relative contribution of drinking water and other pathways (such as food, swimming/recreational, and other environmental exposures) to the total disease risk from all sources. This could also include the development of microbial bioaccumulation factors (BAFs) for organisms that can accumulate human pathogens and are eaten by humans raw or partially cooked (e.g., shellfish). This could be based on an understanding of “disease ecology” (e.g., consider all exposures that result in a given health endpoint) rather than on common assumptions that tend to simplify that understanding (e.g., exposure via a single pathway).

Risk Characterization

- Developing methods to address **cumulative risks from exposure to multiple pathogens** and to pathogens and certain chemicals.
- Developing additional methods for considering possible **lifetime, cumulative risk** from exposure to one or more pathogens.
- Framework for conducting **community-based** cumulative risk assessment.
- Developing methods for estimating risks of **chronic sequelae**.
- Developing methods for comparing risks among different pathogens and different exposures (**comparative risk**). Common metrics that provide a basis for such comparisons (e.g., to compare *Vibrio vulnificus* and *E. coli* O157:H7) should be explored. The use of DALYs is one method for comparing risks.
- Conducting research on the appropriate use of **adjustment factors for microbial risk assessment**. The circumstances for using such factors and the criteria to determine the magnitude of the factors and where they could be applied should be considered.
- Developing additional **model validation methods** to compare the results of the risk assessment with “reality.” If few data exist for this comparison, after the risk assessment is conducted endpoints should be monitored so the model can be validated in the future.
- Further developing **qualitative assessment methods**, because quantitative data are not always available.
- Improving the application of **risk assessment as a predictive tool in developing prevention strategies**.
- Further developing methods and models for incorporating information on **secondary transmission**.
- Further developing methods and models for **incorporating information on immune status**. For some dose-response datasets where infection is the endpoint, this may be difficult because immunity affects illness rather than infection (e.g., as observed for *Giardia*).

General research needs to improve MRA include the following:

- more information on mechanisms of infection and virulence factors;
- data on variation among different hosts and pathogens;
- data on the effect of environment on pathogen growth, survival, and death;
- data from longer time frames in order to account for longer-term weather cycles (e.g., el Niño);
- data on changing land use patterns advancement
- improved sampling, detection, quantification methods, and viability/infectivity assays; and
- Continued development of a thesaurus or lexicon of risk assessment terms to facilitate the evolution of terminology.

APPENDIX F. Exposure Analysis Annex

Table F.1. Tools and Databases for Evaluation of Occurrence

| Tools | Reference |
|--|---|
| USDA agricultural runoff models (hydromodels) | http://wmc.ar.nrcs.usda.gov/technical/WQ/modeldesc.html (list of information and links for further info. on 15 Natural Resources Conservation Service [NRCS] models related to water and agriculture) |
| USDA ARS HYDRUS models | http://www.ars.usda.gov/Services/docs.htm?docid=8910 Simulates water flow and solute transport in a two-dimensional variably-saturated medium (Windows-based graphical user interface) |
| EPA Basins Program | http://www.epa.gov/OST/BASINS/ A multi-purpose environmental analysis system that integrates a geographical information system (GIS), national watershed data, and state-of-the-art environmental assessment and modeling tools into one convenient package. Download BASINS 3.1 at http://www.epa.gov/waterscience/basins/basinsv3.htm . (This release includes additional links to water quality models as well as a new data user interface tool with access to national data layers.) |
| EMPACT Study | General information is available at: http://www.epa.gov/nerl/news/forum2003/water/brenner_poster.pdf and http://www.epa.gov/ORD/NRMRL/pubs/625r02017/beaches_html/chapter1.html . (Location-specific EMPACT studies are available on the Internet.) |
| EPA Information Collection Rule (ICR) and Supplemental Surveys (provides summarized data on <i>Cryptosporidium</i> only) | Overview with links for further information is available at: http://www.epa.gov/enviro/html/icr/ and http://www.epa.gov/safewater/icr.html . |
| AWWARF report on effects of meteorological events (<i>Cryptosporidium</i> only) | http://awwarf.org/research/topicsandprojects/execSum/488.aspx . “Rainfall events and other watershed perturbations, especially those during the spring runoff, pose the greatest risk for causing waterborne cryptosporidiosis.” |
| Safe Drinking Water Information System (SDWIS) | SDWIS—Federal (SDWIS/FED) version is EPA’s national regulatory compliance database for the drinking water program. It includes information on the nation’s 170,000 public water systems and violations of drinking water regulations. <ul style="list-style-type: none"> • Access Drinking Water Information Online (through summary pivot tables, Envirofacts, or direct connection to the mainframe) http://www.epa.gov/safewater/data/getdata.html. • SDWIS/FED Website (information for users who work with the database) http://www.epa.gov/safewater/sdwisfed/sdwis.htm. |
| Statistical method: MCMC simulation for modeling environmental pathogen concentrations in natural waters | Crainiceanu et al. (2003) “Modeling the United States national distribution of waterborne pathogen concentrations with application to <i>Cryptosporidium parvum</i> .” |

APPENDIX G. Human Health Effects Annex

G.1 Choosing a Model for Microbial Dose-Response

Given the availability of dose-response data, statistical methods allow fitting of any mathematical relation with suitable properties. Discrimination between models on an empirical basis using only experimental data, however, is not usually possible. The choice of dose-response model should also be based on insights into events leading to the response (Teunis and Havelaar, 2000). Several models may fit available data in a statistically indistinguishable manner, but provide wide-ranging estimates for the risk at an extrapolated low dose. Although it is theoretically possible to test the potential appropriateness of different dose-response functions against outbreak data, outbreak reports with good quality information about the dose and attack rate are rare.

Haas et al. (1999) pose several basic questions for the risk assessor when choosing a model to estimate dose-response, including the following:

- Assuming a given dose-response model, what are the best parameter estimates using the experimental data available?
- How is it determined which set of plausible models provides the best fit to the data?
- Is the best-fitting model adequate or is there still a significant amount of unexplained variance?
- What is the uncertainty in the parameters estimates of a particular model with the available data?
- Are the results from two or more data sets adequately describable by a common set of dose-response parameters?
- How can lack of fit be explained?

Because the specific mechanisms of infection are not well-characterized, dose-response models are typically selected or rejected on the basis of their goodness of fit to the available data. A detailed discussion on computing goodness of fit for dose-response models is beyond the scope of this MRA Protocol. Interested readers are referred to Haas et al. (1999), Teunis and Havelaar (1999), and FAO/WHO (2003). Likelihood methods have traditionally been preferred; the general procedure involves optimizing the dose-response parameter values so as to minimize the deviance of the predicted response from the actual response observed for a specific dataset. The optimized deviance is then compared to a chi-squared distribution, and acceptability of the model is rejected if the optimized deviance is in excess of a specified upper percentile of the distribution (Haas et al., 1999). Multiple models can be compared for goodness of fit and parsimony using statistics such as the Akaike Information Criterion (AIC) (Akaike, 1981; FAO/WHO, 2003). Bootstrap methods (e.g., repetitive Monte Carlo sampling directly from the data or from data summary distributions) may also be used to fit dose-response models (Haas et al., 1999).

Regardless of whether likelihood-based, Bayesian, or bootstrap methods are used, the dose-response modeling results should provide estimates of the statistical distributions and confidence limits of the dose-response parameters, as well as confidence limits on predictions of individual risk resulting from the defined exposures. This information can then be used to assess the overall uncertainty in risk predictions and in sensitivity analyses to identify the most important variables contributing to the uncertainty.

Both the exponential and beta-Poisson models predict that the change in risks with dose level will be linear at low doses. The models differ, however, in how rapidly they become linear at low doses for a given set of parameter values. More complex models, such as the beta-Poisson, tend to predict greater low-dose risks than the exponential model. When data from a variety of volunteer studies were used to test the best fit between exponential and beta-Poisson models, the beta-Poisson model more often provided an improved fit because of the incorporation of variable distribution of pathogen-host responses. The beta-Poisson model is often preferred for use in extrapolating experimental dose-response data to low doses, as for water or food contamination risk assessments.

G.2 Non-Threshold Assumption

Although apparent “thresholds” (doses below which no adverse effects are observed) are seen in some dose-response studies, for the most part, these observed thresholds are due to a lack of statistical power to observe low probability events rather than the presence of true thresholds (Haas et al., 1999).⁴¹ This conclusion is consistent with the theoretical basis for dose-response models, as described above. There could be situations (involving pathogens whose distribution in space is “patchy,” where pathogen concentrations vary over time, and where pathogen survival is related to its own population density) where threshold infectious levels (as measured by “average” pathogen concentrations) might exist. While EPA believes that it is prudent to assume that, for the exposure concentrations and risk levels of concern, there is no exposure increment that is not associated with an increase in infection or illness risk, the Agency will continue to review the results of ongoing epidemiological studies for additional insights on the possible existence of practical or actual thresholds.

G.3 Sources of Uncertainty in Dose-Response Models

Risk models for pathogens can generate estimates of the uncertainty associated with the dose-response parameters (e.g., the r parameter), and upper confidence limits can be derived that take into account the numbers of experimental animals and the degree of variability in the observed responses. Two important sources of uncertainty for microbial dose-response are discussed below.⁴²

⁴¹ Although virulence factor expression and GI tract colonization have been demonstrated to be influenced by quorum sensing in some pathogens (e.g., *Vibrio cholerae*; Zhu et al., 2002), the relevance of this phenomenon in determining possible thresholds of infection is not well known.

⁴² Note that in this context variability is a source of uncertainty.

Pathogen Variation—Variation Among Strains and Within a Given Strain

An important difference between microbial and chemical risk assessment is that different strains or isolates of a pathogen may differ in infectivity, even if all other factors such as exposure scenario and host susceptibility are the same (*Cryptosporidium* [Teunis et al., 2002] and *Listeria* [FDA/USDA, 2003] are good examples). A chemical compound has reasonably consistent, reproducible toxicity if exposure scenarios and test subjects remain consistent. Microbes can have a great degree of variability (in infectivity, virulence, environmental survival) within strains. Even for “pure isolates,” batches can differ. For microorganisms that cannot survive freezer storage or do not maintain their genetic integrity in freezer storage or tissue culture, *in vivo* passage in animals is required to maintain stocks. Even if the starting inoculum for a dose-response study is clonal, mutations will occur that may impact subsequent pathogen characteristics. When the starting inoculum is not clonal, which is most often the case, the subpopulation ratios within an individual host can differ from other hosts receiving the same inoculum. The subpopulation ratios may also vary as infection progresses, so collecting pathogens from a host on one day may not yield the same pool of pathogens as collecting on another day. In addition, some pathogens are not amenable to storage or maintenance in laboratory settings and must be collected from the environment for each experiment. Even though the challenges of properly characterizing and controlling pathogen variability in experimental research settings are considerable, the challenges are even greater for epidemiological studies. Often the identity of the strain to which the population of concern is exposed is not known. Strain stability through time and variability among strains are sources of uncertainty that should be discussed in the dose-response analysis. In addition, the effects of the strain(s) of microorganisms present in environmental media may not be well-characterized for any given exposed population.

Media and matrix effects for the delivered dose (e.g., drink, food, capsule) can be important for pathogen viability and can impact the ability of the pathogens to move through the stomach and reach target cells in the GI tract. This is an additional source of variation, although is mainly an issue between studies.

Host Variation

Another difference between chemical and microbial risk assessment is the difference in the sources and magnitudes of variability in individual human or test animal responses to a specific exposure. For chemicals, genetic polymorphisms affecting metabolism or sensitivity and physiological differences related to age and gender tend to account for most of the inter-individual variability in response. For microbes, many of the same factors affect the magnitude of risk; for example, there is a known relationship between a specific genetic variation and human host susceptibility to norovirus infection (Moe et al., 2002). For microbes, previous exposure history can affect the immunological status of an exposed population, resulting in large variations in individual sensitivity. In addition, nonspecific immune responses that are innate and do not rely on previous exposure to a pathogen to function (e.g., phagocytic ability of macrophages) can also vary among hosts. Some of these nonspecific responses are not as effective in newborn and infants compared to adults, but some function at an adult level at birth and may be naturally decreased in the elderly. Behavioral or genetic differences that influence

GI tract factors (e.g., pH, digestive enzymes), such as diet and medicines may also influence variation in host response. However, these behavioral host factors do not have general influences among different pathogens, so can only be considered on a case-by-case basis. Although some host behaviors that influence GI tract factors may be linked to pathogenesis in individual case studies, population-wide quantitative data sufficient for inclusion in QMRA modeling do not exist.

All of the above sources of uncertainty can be addressed to some extent when deriving dose-response models for microorganisms. For example, EPA has developed dose-response models for mixed exposures to two different strains of *Cryptosporidium* (Messner et al., 2001) while FDA has developed estimates of the distribution of infectivity of *Listeria monocytogenes* using data from nine different strains (FDA/USDA, 2003). Similarly, Latimer et al. (2001) used a weighted composite dose-response model to account for varying infectivity of *Salmonella* strains. In addressing the variability among hosts, Teunis et al. (2002) noted that the probability of any single oocyst of *Cryptosporidium* to cause infection appears to depend on pre-existing immunoglobulin (IgG) levels of the exposed individuals. Based on this observation, they built an IgG dependence into the dose-response relation and noted that this modification could be easily applied in quantitative risk analysis. As discussed above, meta-analyses, including Bayesian and MCMC approaches, can be used to derive composite, probabilistic dose-response functions from data from multiple studies and strains.

G.4 Selection of Dose-Response Data

Gathering a body of data to develop dose-response estimates for modeling microbial risk involves evaluating different types of studies that examine the relationships between specific pathogens and health outcomes in either animals or humans. In the study of infectious disease, human data in the form of epidemiological studies are more abundant than what is available for chemical risk assessment, which typically relies more heavily on animal toxicology studies. These must then be extrapolated to the human experience. This is largely due to the fact that the acute disease responses most commonly associated with pathogens are easier to track epidemiologically than the long-term effects associated with chronic exposures to toxic chemicals.

The main variables involved in creating dose-response models for waterborne pathogens are virulence (the ability of the pathogen to produce illness), infectivity (the ability of the pathogen to colonize in the host), and host susceptibility. The term *susceptibility* has been used by various disciplines and has had several different definitions (see Parkin and Balbus, 2000). Infectious disease epidemiologists have defined susceptibility as a quantal (“all-or-none”) state, depending on whether an individual has pre-existing immunity to a specific infectious agent. The term *susceptible* has also been used to describe a predisposition to diseases that have a genetic basis, such as forms of cancer that can run in families. For the purpose of this MRA Protocol, host susceptibility is defined as the capacity of the host to defend against the pathogen. Variability in host susceptibilities results in some populations being more susceptible than others; thus, the available data must be evaluated to determine which populations were addressed by the study methodology and to what degree. Often, however, data are only available for a limited group; for example, in the case of volunteer feeding studies—a group of healthy adults.

Understanding the characteristics of data sources is important to the selection and interpretation of data. Risk assessors often use data differently than how it was originally intended. The properties of the data will depend on the perspective of the researchers generating the data (e.g., experimenter versus epidemiologist). Therefore, knowledge of the source and original purpose of the available data sets is important in the development of dose-response models. The following sections briefly summarize the strengths and limitations of each of several classes of data sources.

G.4.1 Animal Studies

In chemical risk assessment, animal studies are used extensively. In the case of microbial infection, it is difficult to develop dose-response animal models for most pathogens because of their specificity to humans—but some do exist. Several effective models (e.g., primates, pigs) can be expensive and may be limited in the number of animals that can be used per dose group. However, when available, animal studies can be used to overcome many of the logistical and ethical limitations that are associated with human-volunteer feeding studies.

Although there are advantages of using animal studies, a direct correlation between the dose-response or disease symptoms in humans versus animals seldom exists. Often, physiological differences between humans and animal species lead to substantial differences in their dose-response relationships. In toxicology, the dose-response parameter assumes a relationship between body mass and effective dose, so that an equivalent dose is measured per kilogram of body weight. Pathogens typically do not have a similar weight-to-effect relationship. Another issue relates to exposure histories. Experimental animals involved in infectious pathogen exposure studies are typically immunologically naïve, in other words, they have not been previously exposed to the organism under study—unless the study has been designed to evaluate the effects of previous exposures. In addition, animals that have been immunologically altered to increase their susceptibility (e.g., gamma knock-out mice) are sometimes used to obtain dose-response data. In some cases, unaltered animals would not readily be infected by the pathogen of concern. Humans, of course, are exposed to a wide array of microorganisms every day from their birth and their immunity profile plays an important role in the likelihood of becoming infected or ill due to pathogen exposure.

The main benefit of using animal studies is the ability to measure the pathogen dose and define a clear health endpoint. Different strains of the same pathogen can be tested in different sets of animals and used to create a range of dose-response estimates. It is important to use pathogen strains that are identical or closely related to the strain of concern for humans, because, even within the same species, different strains of pathogens may have different characteristics that cause variation in their abilities to infect the host and cause illness. Also, because animals can be studied throughout their relatively short lifetimes, animal models can be helpful in characterizing dose-response at different ages. Special populations, such as neonates, pregnant females, or immunodeficient animal populations can be used to study susceptible populations that could not be included in human study protocols for ethical reasons. A number of animal models are relatively feasible, which increases the potential for testing a variety of strains with more replicates and at more doses. However, it is important to emphasize that not all human

pathogens can infect animals.

Animal data should be evaluated for its relevance to the human condition and also the applicability of animal surrogates dose-response models.

Human outbreak or surveillance data should be used to corroborate any observations made in surrogate animals. Depending on the quality and amount of human data available for this validation, an infectious dose based on animal data can be modified to account for various factors, such as strain variation, host susceptibility, and differences in susceptibility of laboratory animals in a controlled environment versus humans in an uncontrolled environment. Although the methods for modifying dose-response of pathogens are not highly developed within the MRA field, some methods do exist. For example, the FDA used a combination of experimental animal studies in mice and epidemiology studies to develop an MRA for *Listeria monocytogenes* (FDA/USDA, 2003). First, the dose-response function was developed from the mouse model, then the dose was adjusted for virulence and host susceptibility and the mouse model was adjusted using the CDC estimates of annual death rates to calculate the dose-response function for humans (deaths/serving).⁴³

G.4.2 Human Studies

Epidemiology has been defined as the study of the distribution and determinants of disease and health in specific human populations (Last, 1995). Although epidemiological studies allow for direct observations of humans, quantitatively characterizing the exposure in observational studies is difficult because the pathogen dose is difficult to ascertain. Animal studies are more precise than epidemiological studies, because the pathogen dose and the resulting response can be controlled and measured; however, pathogens can be species-specific, and to obtain meaningful data, the animal infection model must parallel the human infection model as closely as possible.

In addition to epidemiological studies, volunteer feeding studies are occasionally used to evaluate microbial dose-response relationships. In volunteer feeding clinical studies, the dose is measured and administered in a controlled manner that is similar to how experimental animal studies doses are controlled. Limited dose-response data may also be available from outbreak investigations. For example, the Chicago Department of Public Health, with support of the FDA, has developed a questionnaire for use by outbreak investigators to obtain possible dose-response information, such as what serving size was consumed during a foodborne disease outbreak.⁴⁴

Volunteer Feeding Studies

Researchers have conducted studies examining the health effects of exposing humans to pathogenic microorganisms under controlled conditions for decades. The fact that it only takes a dose as small as 10 *Giardia* cysts to cause infection was discovered by Rendtorff (1954) using prison volunteers. Recent volunteer feeding studies that have been particularly informative for risk assessors have been published by Chappell and colleagues (e.g., DuPont et al., 1995; Okhuysen et al., 1999). Data from these studies have been used in dose-response modeling for

⁴³ <http://www.cfsan.fda.gov/~dms/lmr2-4.html#Modeling-Mice>

⁴⁴ http://www.foodrisk.org/dose_resp.htm

risk assessment for *Cryptosporidium* (see Messner et al., 2001 and Teunis et al., 2002). Use of volunteers is the most direct means of acquiring data that relates an exposure to a microbial hazard with an adverse response in human populations.

Although controlling the conditions under which human dose-response effects can be observed is ideal, ethical and economic limitations severely restrict the use of volunteers. These studies are generally conducted using only healthy individuals between the ages of 18 to 50 years, so they do not examine at-risk (more susceptible) subpopulations. Life-threatening pathogens are obviously not appropriate for volunteer studies. Typically, the studies investigate a limited number of doses of a few strains, with a limited number of volunteers per dose. The dose ranges are generally high enough to ensure a response (i.e., infection and/or illness symptoms) in a significant portion of the test population. The low-dose exposures that are often of most interest to risk assessors are not included in feeding studies because a small group of healthy individuals (i.e., those assessed in human volunteer studies) does not represent how a large mixed health population would respond to low-doses of exposure to a particular pathogen. Because of the characteristics of volunteer studies, their results are generally biased toward low pathogenicity and low host susceptibility, whereas outcomes from outbreak studies (discussed below) are biased toward high pathogenicity and high host susceptibility. Therefore, using data from both types of studies is desirable, if available (Teunis et al., 2005).

When evaluating the experimental design of human volunteer studies, the following issues need to be considered (FAO/WHO, 2003):

- How was the dose measured (both units of measurement and the procedure used to measure a dose)? Include analytical accuracy and precision, that is, to what degree does the assay method detect all of the viable and infectious organisms?
- How did the units in which a dose was measured compare with the units of measurement for the pathogen in an environmental sample?
- Total units measured in a dose may not all be viable units or infectious units (e.g., some dead or non-infectious organisms were counted by the assay).
- Volunteers given replicate doses may not all have received the same amount of inoculum.
- How was the inoculum administered? Did the protocol involve simultaneous addition of agents that alter gastric acidity or promote the passage of microorganisms through the stomach without exposure to gastric acid? (e.g., administered in a matrix that influences GI tract factors)
- How was the volunteer's immunological status assessed (serum antibodies may have dropped to undetectable levels or the volunteer may have been previously infected with a similar pathogen that may not be detected by the serological test)?
- How was infection defined?
- How was illness defined?
- When comparing the dose-response of more than one organism, the same endpoints must be used (e.g., infection versus illness).

G.4.3 Outbreak Investigations

The major limitation to outbreak investigation data is that investigators often collect a narrow

range of information because their main objective is to rapidly identify the vehicle and prevent additional infections. The mode of transmission varies for pathogens depending on a number of factors. All but a few waterborne pathogens can be transmitted by other routes including foodborne and person-to-person transmission. Thus, an outbreak investigation is necessary to determine the mode of transmission of primary cases and possibly in secondary cases. Outbreak investigations can often provide valuable information about the etiologic agent. A pathogen's ability to produce an outbreak depends on specific characteristics such as ability to survive in the environment and rate of growth or die off, potential to cause disease at a given dose, most likely transmission route, and capacity to spread through person-to-person contact. Therefore, studying the details of outbreaks can help risk assessors develop an exposure-response profile for specific pathogens and data from outbreaks can be an important validation for dose-response models and animal models. Key epidemiological calculations are used to investigate outbreaks, including attack rate and incubation period.

An attack rate is defined as follows:

$$\frac{\text{Number of people at risk who develop a certain illness}}{\text{Total number of people at risk}}$$

The attack rate is useful to compare the risk of disease in groups with different exposures. The attack rate can be calculated for a specific exposure; for example, the number of people who developed AGI after swimming in a lake divided by the total number of people who swam in the lake. This would be the primary attack rate—the primary cases who got sick because of their exposure to the water. The secondary attack rate is the number of people who get sick after being exposed to a primary case. It measures the secondary transmission; that is, the incidence of person-to-person spread of the pathogen and subsequent illness. Attack rates may be based on signs and symptoms rather than laboratory-confirmed cases, and this should be considered when assessing the attack rate. They can be underestimated; for example, if the total exposed population is large and not all of the cases are detected. The reported case findings depend on the investigator's case definition. Case definitions may be based on clinical symptoms, on laboratory data, or on a combination of the two. An efficient approach is to choose a clinical case definition and validate it with a sample of cases that are confirmed by laboratory tests.

Data from an outbreak where the cause is confirmed can provide risk assessors with information on the pathogen that was responsible for the outbreak, particularly the range of illness that a pathogen can cause and host characteristics that may increase or decrease the risk. The strain responsible for the outbreak may not have been isolated, so specific strain information may not be available. In addition, the actual dose received may be difficult to approximate because the best exposure estimate is subject to error based on the quality of the water samples or consumption information. When the outbreak is foodborne, it is often possible to sample the food items under suspicion. If the suspected vehicle is processed food, it may even be possible to track down other samples based on lot number and other distribution identifiers. However, when the outbreak is waterborne, it may be nearly impossible to obtain a useful sample to test. In the 1993 cryptosporidiosis outbreak in Milwaukee, WI, by the time the outbreak was identified and the municipal water supply was suspected as the source, the conditions leading to the contamination had dissipated. In a novel attempt to better characterize the outbreak

conditions, public health investigators used ice cubes frozen during the time of suspected contamination to help identify the number of *Cryptosporidium* oocysts in the drinking water (MacKenzie et al., 1994).

If actual levels of food or water contamination can be measured, an outbreak that is characterized by a low attack rate in a very large population may provide an opportunity to define the host-response to very low doses of a pathogen (Teunis et al., 2004). Even when obtaining samples from the suspected pathogen source is not possible, dose-response relationships may be observed as variation in health outcomes with changes in relative dose, assuming dose affects symptom severity and not just risk of infection.⁴⁵ Epidemiological observations can help identify susceptible subpopulations; for example, for higher attack rates among persons who consumed more of the implicated water source or swam more often in the contaminated lake, or variation in symptom prevalence and complications.

The investigator of an outbreak typically goes through the following steps (adapted from Gordis, 2000):

1. Define the epidemic
 - a. Define the numerator (cases)
 - i. Clinical features: Is the disease known?
 - ii. What are its serologic or cultural aspects?
 - iii. Are the causes partially understood?
 - b. Define the denominator: What is the population at risk of developing disease?
 - c. Calculate the attack rates
2. Examine the distribution of cases by the following:
 - a. Time
 - b. Place (look for time-place interactions)
 - c. Common sources of exposure
3. Look for combinations (interactions) of relevant variables
4. Develop hypotheses based on the following:
 - a. Existing knowledge of the disease
 - b. Analogy to diseases of known etiology

The investigation should determine the source of the exposures and characterize the magnitude and duration of the outbreak as well. Limitations of such studies include the technical difficulty in detecting and quantifying the causative organisms in contaminated food or water; recall bias regarding estimates of how much food or water they consumed; inadequate information about the health status of the exposed population, including the number of individuals exposed who did not get infected or those who developed asymptomatic infection; and uncertainty in the size of the total exposed population (FAO/WHO, 2003). An example of a risk assessment that used data from 33 different *Salmonella* outbreaks was published in 2002 (WHO/FAO, 2002).

⁴⁵ Note that outbreak situations are different than background endemic levels of pathogens. For example, the LT2 *Cryptosporidium* risk assessment only modeled low doses (non-outbreak situations) and therefore made the assumption that dose was related only to infectivity and not to subsequent symptom severity.

G.4.4 Health Surveillance Data

National and international organizations compile health statistics for infectious diseases, including those that may be transmitted by food and water (Doyle et al., 2002; Wheeler et al., 1999). Such data are critical to adequately characterize microbial hazards. In addition, surveillance-based data have been used in conjunction with food survey data to estimate dose-response relationships. The collection of surveillance data is highly dependent on the sophistication of the surveillance system, and even then, analyzing such aggregated data requires making many assumptions that increase the uncertainty of the results. Many factors affect the likelihood that a waterborne outbreak will be recognized, investigated, and ultimately reported. In general, outbreaks with high attack rates (i.e., incidence of infection in a group observed during an epidemic) or a large number of cases of illness associated with severe symptoms in a state/locality that has had previous waterborne outbreaks are more likely to be recognized (NRC, 2004). Outbreaks that are more likely to be missed by surveillance efforts include those that have low attack rates or are associated with mild and/or common illness symptoms. Those caused by agents that are not easily detected or identified (e.g., viruses) are also likely to go unreported. Regional surveillance data was used in a New York City risk assessment of cryptosporidiosis due to drinking water consumption (Makri et al., 2004).

Outbreak data are useful for identifying deficiencies in the provision of safe drinking and contaminated recreational water, evaluating the adequacy of regulations for water treatment, and monitoring water quality, but outbreak data have limitations. State, territorial, and local public agencies are responsible for detecting, investigating, and reporting outbreaks. Because reporting is often voluntary and passive, and varies by agency, the reported data is widely known to underestimate the incidence of outbreaks (NRC, 2004). The extent of underestimation varies widely from locale to locale and is unknown overall; therefore, the statistics reported in CDC's periodic Surveillance Summaries (e.g., CDC, 2004) represent only a portion of the burden of illness associated with drinking water exposure. In addition, the surveillance information does not include endemic waterborne disease risks. Nonetheless, this voluntary reporting system, where outbreaks are the unit of measure, has increased our knowledge of waterborne diseases—despite underreporting. Consequently, waterborne disease outbreaks often are inconsistently detected and reported, leading to difficulty in ascertaining the total incidences of illness resulting from contact with contaminated recreational waters or drinking water (Payment and Riley, 2002).

If available, the surveillance summaries identify the etiologic agents causing recreational and drinking water disease outbreaks and often the sources of the contamination. However, the etiologic agents are often not identifiable. In the 2004 CDC report on recreational water outbreaks, 81.5% of the 65 outbreaks reported for years 2001 to 2002 had information on the etiologic agent available (CDC, 2004). Of the 30 outbreaks involving gastroenteritis (AGI) during the same period, 23.3% were of unknown etiology. In some cases, biological specimens are either unavailable for testing, or an agent was not detected in the specimens provided. Microbial testing methods have improved over the years, especially for viruses, so the percentage of outbreaks with unknown etiologies has decreased. For example, in 1985, half of the reported outbreaks related to drinking water were of unknown etiology and no viral outbreaks were identified. Improved laboratory methods also affect the ability to detect the source of

contamination. Because water quality data are not always reported as part of an outbreak, the waterborne disease outbreak statistics cannot always be linked to information about whether water quality standards were exceeded prior to the outbreak or whether outbreaks occurred in water bodies meeting the standards.

Like outbreak data, annual surveillance statistics provide a way to check the plausibility (informal reality check) of MRA models. The effectiveness of dose-response models is typically assessed by combining them with exposure estimates and determining if they approximate the annual disease statistics for the hazard. Using annual disease statistics in modeling dose-response and exposure estimates implicitly includes the entire population and the wide variety of factors that can influence the response. Another benefit is that surveillance databases often have sufficient detail to analyze special subpopulations such as the elderly or the immunocompromised. It should also be noted that outbreak data may also provide information on factors that promote secondary spread (e.g., a behavioral component) or situations where secondary spread drives the outbreak. For example, person-to-person or person-to-environment-to-person can be important for outbreaks due to noroviruses, *Shigella*, and *Cryptosporidium* (Eisenberg et al., 2002, 2003).

The occurrence of endemic (i.e., non-outbreak related) waterborne disease has only recently become a focus of the U.S. government. The 1996 amendments to the SDWA (§1458(d)(1)) require EPA and CDC to collaboratively design and conduct pilot waterborne disease occurrence studies for at least five major communities or public water systems, prepare a report on the findings, and develop a national estimate of endemic waterborne disease occurrence. Thus far, the studies on endemic waterborne disease due to drinking treated water have not established a good correlation between waterborne pathogens, indicators, and adverse human health effects (Colford et al., 2005; NRC, 2004).

The Foodborne Diseases Active Surveillance Network (FoodNet⁴⁶) is the primary foodborne disease component of CDC's Emerging Infection Program (EIP). FoodNet is a collaborative project of CDC, 10 EIP states, USDA, and FDA. FoodNet provides active surveillance for foodborne diseases and related epidemiological studies designed to help inform and educate public health officials regarding the epidemiology of foodborne diseases in the United States. Notably, the FoodNet population survey collects information on recent GI illness and is serving as one source of the incidence rates of GI illness in the U.S. population that are needed to calculate the national estimate of endemic waterborne disease (NRC, 2004). PulseNet (the National Molecular Subtyping Network for Foodborne Disease Surveillance⁴⁷) is CDC's network of public health laboratories that provide an early warning system for outbreaks of foodborne disease using a DNA "fingerprinting" method. The network permits rapid comparison of these fingerprint patterns for several strains of foodborne pathogens (e.g., *E. coli* O157:H7) through an electronic database, provides critical data for the early recognition and timely investigation of outbreaks, thus reducing the burden of foodborne disease. For pathogens that are both foodborne and waterborne, FoodNet and PulseNet may provide information that can assist in microbial risk assessment.

⁴⁶ <http://www.cdc.gov/foodnet/>

⁴⁷ <http://www.cdc.gov/pulsenet/>

G.5 Alternate Dose-Response Models

G.5.1 Empirical Models

To date, the majority of studies in dose-response modeling and microbial risk analysis for waterborne pathogens have employed the exponential and beta-Poisson dose-response models. These models are mechanistic (based on models of biologically-plausible processes), relatively simple, and have provided good fits to virtually all data sets for which they have been applied. Other models have also been proposed and used as components of MRAs, particularly in the assessment of risks associated with food. These alternative models are empirical (i.e., not derived based on consideration of biological processes) and as such, their validity outside the data range for which their parameters are estimated is unknown, and extrapolation with these models is not recommended (Buchanan et al., 2000). In dose-response model selection, preference is given to biologically plausible, mechanistic models such as the exponential and beta-Poisson over empirical models. Other researchers (e.g., Coleman and Marks, 1998) have suggested that the exponential and beta-Poisson models are not substantially different from empirical models. However, this Protocol does not adopt that position because exponential and beta-Poisson models may be derived from basic considerations of the infection process, because the models may be adapted to include other processes within the infection process, and because of the demonstrated success in fitting the exponential and beta-Poisson models to available data.

Buchanan et al. (2000), Moon et al. (2004), Holcomb et al. (1999), and Haas et al. (1999) compared the exponential and beta-Poisson models to various empirical dose-response models proposed for use in dose-response modeling for foodborne pathogens. The empirical models assessed in those studies are summarized in Table G.1. Presentation of these models in this protocol is intended to provide a thorough description of models that have been used. In their comparison of models, Moon et al. (2004) found that three-parameter models did not yield significant improvements in fit over two-parameter models and that among two-parameter models, predictions in the low-dose range were markedly different between models. Buchanan et al. (2000) suggest the development of mechanistic models with consideration of factors important in the infection process as a route to more accurate dose-response models that may be extrapolated outside the range of available data. Holcomb et al. (1999) noted that among the empirical and mechanistic dose-response models compared, only the three-parameter Weibull-gamma model provided goodness of fit for data sets of dose-response data for four different pathogens and that different models predicted very different low-dose response. These observations lead the authors to suggest that continued dose-response model development and evaluation is necessary.

Another model, not included in Table G.1 because its origin and functional form differ substantially from those models in the table, was recently proposed by Brynestad and Braute (2008) and subsequently applied by Nauta et al. (2009). The model is termed a “sigmoidal model” and is suggested as an alternative to the beta-Poisson or the hazard function model proposed by Teunis et al. (1999) for the predicted incidence of illness given infection. Nauta et al. (2009) suggest that the beta-Poisson and exponential models are valid for predicting rates of infection but not for rates of illness. This assertion notwithstanding, the exponential and beta-Poisson models have been used in many cases for development of dose-response models in

Table G.1. Empirical Dose-Response Models

| Model | Equation | Parameters |
|-------------------------|--|---|
| Weibull-Gamma | $P(d) = 1 - (1 + d^b / \beta)^{-\alpha}$ | Three parameter model: b, α, β . |
| Weibull | $P(d) = 1 - \exp(-ad^b)$ | Two parameter model: a, b |
| Gompertz ¹ | $P(d) = 1 - \exp[-\exp(a + b f(d))]$ | Two parameter model: a, b $f(d)$ denotes a transformation (e.g., log) |
| Log-normal ² | $P(d) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{(\ln d - \alpha) / \beta} \exp(-\frac{1}{2}t^2) dt$ | Two parameter model: α, β |
| Log-logistic | $P(d) = 1 / \{1 + \exp[-(\ln d - \alpha) / \beta]\}$ | Two parameter model: α, β |
| Exponential-Gamma | $P(d) = 1 - \exp(-\gamma d) / (1 + d^b / \beta)^\alpha$ | Three parameter model: α, β, γ |
| Weibull-exponential | $P(d) = 1 - \exp(-\alpha d^\gamma) / (1 + d^\gamma / \beta)$ | Three parameter model: α, β, γ |
| Shifted Weibull | $P(d) = \begin{cases} 1 - \exp\{-[(d - \alpha) / \beta]^\gamma\} & d \geq \alpha \\ 0 & 0 \leq d < \alpha \end{cases}$ | Three parameter model: α, β, γ |

¹ When the function f is the natural log transformation of dose, this models is referred to as an “extreme value” model in Moon et al. (2004).

² Referred to as the log-probit model in Haas et al. (1999).

which illness is an endpoint. Rather than using a biological basis for model selection, Brynstead and Braute (2008) selected the sigmoidal model based on its simplicity and ability to incorporate expert judgment (specifics of which are not provided in this Protocol) and suggest that these bases make the model an improvement over other dose-response models for predicting response at low dose. Their dose-response model is given by

$$p(d) = (\max - \min) \left(1 + 10^{\log(IID_{50}) - d} \right)^{HS} \tag{G-1}$$

where HS is the Hill slope, given as

$$HS = \frac{\log[q / (100 - q)]}{\log(IID_q / IID_{50})} \tag{G-2}$$

q is the the chance of becoming ill (selected as 1%), IID_{50} is the median infectious dose at which 50% of the exposed population becomes ill,, and IID_q is the dose at which $q\%$ of the exposed population is expected to become ill. The functional form of the sigmoidal relation is such that the probability of illness rises sharply from a very small value at the dose expected to produce

illness in $q\%$ of the exposed population (1% in the model of Brynstead and Braute). Based on published data, Brynstead and Braute (2008) estimated that IID_{50} and IID_1 were uniformly distributed in the ranges 500-800 and 2000-6000 organisms, respectively. Risk estimates for *Campylobacter* infection related to food preparation appear high for both the Hill slope model and an alternative dose-response model, with the Hill slope model providing a lower, but unrealistic estimate of the number of illnesses. This finding could indicate that current dose-response models over-predict the incidence of illness or that exposure models overpredict the incidence and ingestion of *Campylobacter* in the food chain in Germany. Alternatively, the findings could be an artifact of the high uncertainty inherent to the epidemiological data to which QMRA model results were compared.

In their review of food-borne *Campylobacter* QMRAs, Nauta et al. (2009) compared alternative *Campylobacter* dose-response models (illness endpoint) and observed that the sigmoidal model predicts much lower illness probability at low dose than alternative published models. This observation is consistent with the chosen form of the model. Based on the assumptions of Brynstead and Braute (2008), the sigmoidal illness incidence model reported by Nauta et al. (2009) used a 1% illness incidence as the lower end of the illness dose-response relation, arriving at the following dose-response model:

$$p_{\text{ill}}(d) = \frac{1}{\left(\frac{IID_{50}}{d}\right)^a + 1} \quad [\text{G-3}]$$

where IID_{50} is the dose at which 50% of the exposed population becomes ill, d is the ingested dose and a is given by

$$a = \frac{\left[\frac{\ln(0.99)}{\ln(0.01)}\right]}{\left[\frac{\ln(IID_{50})}{\ln(IID_1)}\right]} \quad [\text{G-4}]$$

The comparison of QMRAs of *Campylobacter* by Nauta et al. (2009) allowed the authors to conclude that the *Campylobacter* dose-response model remains unknown, particularly given potential variations in the ability of different strains of *Campylobacter* to initiate infection or illness, influences that food matrix or other environmental factors may exert on the incidence of illness, and the difference in response for different subpopulations.

Selection of dose-response models requires comparison of fits of the models to data and comparison of fits of more highly-parameterized models with those of models with fewer parameters. When maximum likelihood estimation is used for determining the model parameters, fits are compared on the basis of the deviances at the parameter values providing the best fit of the model to the data. In general, models with more parameters are selected over models with fewer parameters only when the improvement in fit of the model with more parameters over that of the model with fewer parameters is statistically significant.

G.5.2 Threshold Models

Threshold models (i.e., models that assume more than one organism is required to initiate

infection) can be derived under slightly different assumptions than those used to develop the exponential and beta-Poisson dose-response models. Assuming pathogens in an ingested dose are drawn from a homogeneous distribution (Poisson distribution) and each pathogen has an equal, independent probability that it can initiate an infectious focus, the probability of infection by k_{\min} organisms is (Haas et al., 1999)

$$P(\text{infection} | d) = \Gamma(k_{\min}, r d) \quad [\text{G-5}]$$

where r is a parameter of the distribution and Γ denotes the gamma cumulative probability distribution function. This simple threshold model is a two parameter model whose parameters may be determined via standard statistical techniques such as maximum likelihood estimation (MLE). Deterministic models have also been used in evaluation of the potential that components of the infection process can produce complete extinction of pathogens populations before a systemic infection (e.g., establishment of a steady pathogen population *in vivo*) occurs (e.g., Blaser and Kirschner, 1999; Coleman and Marks, 2000). These studies are described in the following section.

G.5.3 Mechanistic and Physiologically-Based Models of Infection

Models of the infection process (i.e., mechanistic dose-response models) may be developed with varying degrees of resolution. These models differ in the components of the infection process that are explicitly modeled and whether they are deterministic or stochastic. Early attempts at developing mechanistic dose-response models focused on stochastic pathogen birth and death processes or on division of the infection process into stages that might be modeled separately. Under the assumption that pathogens divide and are removed (via innate or active immune system processes or other means) at constant rates, μ and λ , Bailey (1964) developed expressions for the probability that an *in vivo* population of size N is realized at time t :

$$p_N(t) = \sum_{j=0}^{\min(d,N)} \binom{d}{j} \binom{d+N-j+1}{d-1} A^{d-j} B^{N-j} (1-A-B)^j \quad [\text{G-6}]$$

and for the probability that the pathogen population reaches extinction at time t :

$$p_0(t) = A^d \quad [\text{G-7}]$$

or as $t \rightarrow \infty$:

$$\lim_{t \rightarrow \infty} p_0(t) = \left(\frac{\mu}{\lambda} \right)^d \quad [\text{G-8}]$$

In equations 1 and 2,

$$A = \frac{\mu [1 - e^{-(\mu-\lambda)t}]}{\lambda - \mu e^{-(\mu-\lambda)t}} \quad [\text{G-9}]$$

$$B = \frac{\lambda [1 - e^{-(\mu-\lambda)t}]}{\lambda - \mu e^{-(\mu-\lambda)t}} \quad [\text{G-10}]$$

Morgan (1980) used equation G-6 to derive an expression for the probability that a single pathogen ($d = 1$) achieves a threshold population at time t :

$$p(n \geq N; t) = (1 - A)B^{N-1} \quad [\text{G-11}]$$

Alternately, the probability that the incubation period, T , is less than a time, t , is

$$p(T \leq t; t) = \frac{(1 - A)B^{N-1}}{1 - \mu/\lambda} \quad [\text{G-12}]$$

Morgan used MLE to fit the dose-response model given in equations G-9 to G-11 to incubation period data drawn from a study of the incubation period of streptococcal sore throat (Sartwell, 1950). Morgan's estimates for growth rate, λ , death rate, μ , and number of organisms present *in vivo* at the incubation time, N , were 0.236/hr, 0.190/hr and 46.51, respectively. Morgan hypothesized that the very low value estimated for N results from neglecting complications such as site heterogeneity, eclipse periods and hosts differing in response due to natural or acquired resistance, age, allergic states, etc.

Williams (1965) derived an expression for the probability of a dose of d organisms achieving a net population of N or more organisms at time t . Note the difference between one organism giving rise to a population of N organisms in time t and d organisms achieving a net population of N organisms at time t . Assuming a constant birth rate, λ , and death rate, μ , Williams showed that the distribution of incubation periods (time to achieve a net population of N organisms) for an inoculum of dose, d , is given by

$$f(\tau) = \frac{\sqrt{d}}{e^d - 1} \exp\left(-\frac{1}{2}\tau - e^{-\tau}\right) I_1\left(2d^{1/2}e^{-\frac{1}{2}\tau}\right) \quad [\text{G-13}]$$

In equation G-13, $\tau = (\lambda - \mu)t - \ln[N(1 - \nu)]$, $\nu = 1 - \mu/\lambda$, and I_1 denotes a first Bessel function of the imaginary argument. The model presented as equation 13 was found to provide an excellent fit to the distribution of incubation periods observed in an outbreak of streptococcal sore throat associated with consumption of contaminated milk, although details of the calculations were not provided.

Brookmeyer et al. (2005) developed a time-dependent dose-response model referred to elsewhere as a competing risks model (Gutting et al., 2008). One of the authors' stated motivations in developing a mechanistic model for *Bacillus anthracis* infection was utilization of available data on infection by *B. anthracis* (spore germination rates, clearance rates, growth rates) in the absence of detailed human dose-response data developed in experimental studies. Assuming a constant risk of spore germination per unit time, ω , and a constant risk per unit time of clearance from the lung, κ , and assuming that spore germination implies systemic infection, Brookmeyer and colleagues showed that the cumulative attack probability for inhalation anthrax may be estimated as

$$F(t) = 1 - \exp\left[\frac{-d\omega}{\omega + \kappa} \left(1 - e^{-(\omega + \kappa)t}\right)\right] \quad [\text{G-14}]$$

Inspection of equation 14 shows that the Brookmeyer competing-risks model yields the exponential dose response model in the limit $t \rightarrow \infty$.

Blaser and Kirschner (1999) developed a deterministic model for *in vivo* pathogen growth, including immune system response. In that study, stocks and flows of five quantities—mucus-living *Helicobacter pylori*, *H. pylori* attached to epithelial cells, concentration of bacterial nutrients released via inflammation, concentration of effector molecules, and host response—were included in a system of ordinary differential equations describing the dynamics of these quantities. In mucus, the conservation equation for *H. pylori* accounted for growth (first-order with respect to nutrient availability), loss due to mucus shedding and migration, and gain due to emigration. The conservation equation for *H. pylori* on epithelial cells included a growth term, a loss term related to sloughing, and terms accounting for immigration and emigration. The authors used their model to explore the importance of the parameters in their model of infection, determining that the parameter that describes the ability of the immune system to respond was the most important determinant of whether there would be extinction (all pathogens are removed from the system) or whether sustained growth occurs and that the bacteria growth parameter had a limited effect on the ability of pathogens to initiate infection but was the most important factor in determining the time required for pathogens to reach a steady population *in vivo*. In subsequent modeling work, Blaser and Kirschner (2007) used a deterministic model to explore infections with slow progression or latent periods, during which there is equilibrium between host response and pathogen population dynamics. Taken together, the two studies by Blaser and Kirschner (1999, 2007) demonstrate the utility of deterministic models in exploring complex infection processes.

Coleman and Marks (2000) developed both stochastic and deterministic models of non-typhoid salmonellosis and used the models to identify factors that influence the shape of the dose-response curve in the low-dose region. In that study the important events occurring in the course of *Salmonella* infection were posited to be survival of ingested bacteria to the target, colonization, engulfment, intracellular survival, migration and multiplication, damage, and AGI. The authors suggested stochastic models for each of these processes and presented an alternative formulation based on a predator-prey framework. As pointed out by Coleman and Marks (2000) for infection by non-typhoid *Salmonella* and also by Levin and Antia (2001) for infections in general, there may be physiological and biological process that do not conform to the assumptions underlying the beta-Poisson or exponential dose-response model, including clumping of pathogens in the ingested dose, quorum sensing, and the possibility that organisms do not exhibit independent action. In the context of MRA model results and results of feeding studies of healthy adult human volunteers, the authors made a case for sub-linearity of response at low dose. The authors note that the potential for sub-linear low-dose response is likely differ between pathogen-host combinations and that additional data such as *in vitro* studies may provide information for parameter selection for mechanistic infection models. Development and validation of additional mechanistic models for infection provides an avenue for evaluating low-dose response.

The inherent variability of host-pathogen processes suggests use of stochastic models for describing *in vivo* processes leading to infection. Allen and Allen (2003) describe Markov-chain and stochastic differential equation models for estimating the pathogen burden *in vivo* as a function of time. Their model of the infection process is relatively simplistic, comprised only of birth and death processes in which birth and death rates may vary with time or pathogen density, but their framework is amenable to inclusion of additional components (e.g., immune system components, pathogens in different states). Based on evaluation of different models for a relatively simple case, the authors concluded that combinations of deterministic and stochastic models offer the greatest opportunity for including relevant features of the infection process in a computationally tractable framework.

Recent dose-response modeling efforts have included development of highly-detailed, physiologically-based models of the infection process. In their recent assessment of anthrax dose-response models, Gutting et al. (2008) outlined the components of a hypothetical physiologically-based biokinetic (PBBK) model of infection and response to aerosols of *Bacillus anthracis*. In the model, the fate and transport of *B. anthracis* spores and vegetative cells is tracked in regions of the respiratory system, in macrophages, in the blood and in lymph nodes. As done by Brookmeyer et al. (2005) in their development of a competing risks model for inhalation anthrax, Gutting et al. (2008) estimate model parameters for use in their biokinetic model using physiological and microbiological data not collected in quantal dose-response studies or epidemiological investigation. However, details of the techniques used for parameter estimation or of the model were not provided in the study by Gutting et al. (2008).

G.6 Use of Bayesian Methods in Microbial Risk Assessment

Bayesian methods are being increasingly used by several researchers in microbial risk assessment to estimate dose-response model parameters. In general, a dose-response function gives the probability of illness or infection as a function of the dose and of several unknown parameters. Experimental data are collected from subjects accidentally (in an outbreak) or deliberately (in a controlled experiment with volunteer human subjects or with animal subjects) exposed to a microbial dose that can be measured or estimated. The numbers of subjects that become infected or ill for each dose level are observed, leading to a binomial likelihood. That is, the probability of n out of N “successes” out of N trials of dose level d , where “success” means illness or infection and the success probability is given by the dose-response function. The “traditional” frequentist statistical approach uses the binomial likelihood only, and chooses parameter values to maximize the likelihood.

In general, if N_i subjects are exposed to a dose D_i , and n_i of them developed infection, then the likelihood for the full population (all dose groups) is given by the following:

$$\prod_{i=1}^{\text{\#doses}} \frac{N_i!}{N_i!(N_i - n_i)!} \times [P(\text{infection} | D_i)]^{n_i} \times [1 - P(\text{infection} | D_i)]^{N_i - n_i} \quad [\text{G-15}]$$

The dose-response function is the function $P(\text{Infected} | D)$, which will depend upon the mean dose D as well as the unknown parameters.

Uncertainty intervals for the frequentist parameters are called confidence intervals. On average, out of 100 95% confidence intervals, 95 will contain the parameter value. To estimate the uncertainty of the estimated dose-response parameters and dose-response function, 95% confidence intervals can be calculated using standard asymptotic theory, valid when the sample sizes (“n”) are large. The asymptotic theory uses the likelihood function (Equation 15) to derive an estimated standard error for each parameter, and the 95% confidence interval can then be estimated as the maximum likelihood estimate plus or minus 1.96 standard errors. The 1.96 is the 97.5th percentile of a standard normal distribution, which applies because for large samples the estimated parameter approximately has a normal distribution.

Alternatively, and preferably for the small sample sizes usually available in microbial risk assessment, a Monte Carlo bootstrap resampling method can be used to estimate the uncertainty by randomly sampling with replacement from the original data and fitting the model to each of the resampled data sets.

Bayesian methods exploit available subjective and related information in addition to the numeric data from the experiment or outbreak. Ideally, the investigator expresses their initial assessment of the unknown parameter distribution, prior to examining the data, by defining a prior probability distribution for the parameters. The prior probability distribution is defined based on subjective information and professional judgment.⁴⁸ Using Bayes’ rule, the posterior probability distribution for the parameters given the data can be calculated. From Bayes’ rule, the posterior distribution equals the prior distribution for the parameters multiplied by the likelihood for the data (given the parameters) and then divided by a normalizing constant. The normalizing constant is the integral of the product of the prior and likelihood over all possible parameter values.⁴⁹ In a Bayesian analysis, uncertainty intervals for the parameters and the dose-response function can be calculated from the posterior distribution as “credible intervals”; a 95% credible interval has a 95% probability of including the parameter value, given the data.

The choice of a suitable prior distribution is crucial and can be controversial. Recent published MRAs have usually had very little subjective information to rely on for choosing a prior distribution and the investigators have chosen a “non-informative” prior distribution to represent the lack of prior information. The researchers have usually published their choice of non-informative prior, but have not usually provided a rationale for their choice over other possible non-informative priors.⁵⁰ For example, Teunis and Havelaar (2000) used a beta-Poisson model,

⁴⁸ Some Bayesian researchers use a more objective approach called the empirical Bayes method that is based on an hierarchical model such that the likelihood depends upon parameters that have distributions depending upon other parameters, called hyperparameters. A frequentist approach, such as maximum likelihood, is used to estimate the hyperparameters and thus estimates the prior distribution without the use of subjective information. At this time, the authors are not aware of any applications of empirical Bayes methods to microbial risk assessment.

⁴⁹ Suppose θ is the vector of unknown parameters, and has a prior distribution with probability density function $f(\theta)$. Suppose the data X has a likelihood given by $g(X | \theta)$, for example, Equation 15. Then the posterior distribution will have a probability density given by $f(\theta) g(X | \theta) / k(X)$, where $k(X)$ is the normalizing constant. This is Bayes’ rule. The normalizing constant is the integral

$$\int f(\theta) g(X | \theta) d\theta, \text{ integrated over all possible values of } \theta.$$

The constant $k(X)$ does not depend upon the parameters although it will depend upon the data X .

⁵⁰ Teunis et al. (2004, 2005, 2008a,b) transformed their parameters using logarithms and logit functions to avoid

described below, and chose the prior distribution for their parameters α and β such that their logarithms (base 10) were assumed to have a wide uniform distribution from -12 to +6 and the parameters were assumed independent. Englehardt (2004), using the same beta-Poisson model, instead chose a joint uniform prior distribution for the parameters α and β . Teunis et al. (2004) also used a beta-Poisson model, but used another non-informative prior, such that $\alpha/(\alpha + \beta)$ is uniform from 0 to 1 and $\log_{10}(\alpha + \beta)$ is normally distributed with mean 0 and standard deviation 10. If the non-informative prior distribution is wide then the posterior probability distribution should not be sensitive to the choice of non-informative prior, which justifies the name “non-informative prior.” However, researchers have used different non-informative priors for the same model, which suggests that the choice of the so-called non-informative prior can impact the results.

In the past, Bayesian researchers were much more limited in their choice of prior distributions because they needed to choose a distribution to make the calculations tractable (a “conjugate” prior), particularly the calculation of the normalizing constant. More recently, with MCMC methods and fast computing methods, the calculations can be easily executed for a much wider variety of prior distributions using Monte Carlo simulation methods.

The MCMC method describes a group of methods used to simulate values from a probability distribution for which direct analytical calculations are difficult, intractable, or inconvenient. Gilks et al. (1996) provide a good description of these methods. Well-validated software packages are available to perform these calculations, including WinBUGS and Mathematica. For Bayesian MCMC analyses, the simulated probability distribution is the joint posterior distribution of the parameters given the data. Thus, at each step of the Markov chain, a vector of parameter values is simulated, rather than a single parameter value. Furthermore, it is unnecessary to know the normalizing constant for the posterior distribution, which is often the most difficult part of the calculation. All that is needed are some constant multiples of the prior distribution and the likelihood. The normalizing constants needed to make the prior and likelihood integrate to one are not needed. A version of the Metropolis-Hastings algorithm (Gilks et al., 1996; Hastings, 1970) is used at each step to simulate from the posterior distribution without knowing the normalizing constant.⁵¹ Instead of being statistically independent, the consecutive values form a Markov chain, so that the statistical distribution for one value depends upon the previous value. Using the MCMC method, the Markov chain has a limiting, stationary distribution, so that after a sufficiently long “burn-in” period the values have the desired

high correlations between the parameters and thus improve the estimation. However, this does not really explain their choice of non-informative prior for the transformed variables.

⁵¹ Suppose that the product of the prior and likelihood is equal to $K \cdot f(\theta)$, where θ is the vector of all the unknown parameters and K is an unknown normalizing constant that will depend upon the data values; that is, for MRA, the numbers of illnesses or infections observed. To obtain the posterior distribution, K can, in principle, be calculated as the reciprocal of the integral of f over the range of possible parameter values, but this calculation is often very difficult analytically. Let $q(\theta, \phi)$ be any chosen proposal distribution, which is a probability density for the next parameter vector ϕ that may depend upon the previous parameter vector θ . If the parameter vector at the previous step is θ , one must first randomly sample a parameter vector from $q(\theta, \phi)$ to obtain a candidate vector ϕ^* . With probability α , one can accept the candidate vector, so that the vector at the next step of the Markov chain is ϕ^* . With probability $1 - \alpha$, one rejects the candidate vector, so that the vector at the next step of the Markov chain is, again, θ . The probability α is calculated as $\min(f(\phi^*) q(\phi^*, \theta) / \{f(\theta) q(\theta, \phi^*)\}, 1)$. Because f appears in both the numerator and denominator, the unknown K cancels out and is not needed. A good choice of the proposal distribution will have high acceptance rates and fast convergence to the stationary distribution.

probability distribution.⁵²

The articles reviewed for this discussion do not specify the details of the Metropolis-Hastings algorithms used. Many Bayesian analysts use the Gibbs sampler, which is a special version of the Metropolis-Hastings algorithm that always has acceptance probability 1, so that a new parameter vector is selected at each step. Instead of jointly updating all the parameters in a single step, the Gibbs sampler simulates each of the parameters in turn.⁵³ An algorithm such as Adaptive Rejection Sampling (Gilks and Wild, 1992) is used to generate samples from the distribution of each parameter without needing to calculate the normalizing constant.⁵⁴

Bayesian modeling has been used by MRA researchers in various ways. Several authors have used both Bayesian and frequentist (likelihood-based) methods (Messner et al., 2001; Teunis and Havelaar, 2000). Often the frequentist approach is used to provide maximum likelihood estimates of the dose-response function and the Bayesian approach is used to calculate uncertainty intervals (e.g., 80 or 95% credible intervals for the parameters or the dose-response). The frequentist likelihood ratio test is used to compare different dose-response models. Several approaches use the mode of the Bayesian posterior distribution to select the dose-response function (Teunis et al., 2004, 2005, 2008a,b). The posterior mode is given by the parameters that maximize the posterior probability, defined as the product of the prior and the likelihood; it is again not necessary to calculate the normalizing constant.

Engelhardt and Swartout have published several papers (Engelhardt, 2004; Engelhardt and Swartout, 2004, 2006, 2008) advocating the use of the predictive Bayesian approach, which is the unconditional dose-response probability, calculated as the integral of the posterior

⁵²A burn-in period of about 5000 steps is usually sufficiently long that the stationary distribution has been reached; various convergence tests can be used to assess convergence. Values generated during the burn-in period are discarded, and one usually selects every k^{th} value after the burn-in period for some suitably large k (e.g., 10, 20, 100) so that the remaining “thinned” sequence of values are approximately independent. Thus, the thinned values after the burn-in period can be treated as if they were a random sample from the given probability distribution.

⁵³ Suppose that there are n unknown parameters in the posterior distribution. Instead of generating a new multivariate vector of n parameters from a joint distribution, the Gibbs sampler generates each parameter in turn from the univariate “full conditional” distribution of that parameter given the values of all the other parameters and the data. Thus, each Markov chain step becomes a sequence of sub-steps where the n parameters are scanned in turn and the m^{th} parameter value is randomly selected from the conditional distribution of the m^{th} parameter given the data and the most current values of the remaining $n-1$ parameters (i.e., the values of the first $m-1$ parameters from the current scanning steps and the values of the last $n-m$ parameters from the previous Markov chain vector). An algorithm such as Adaptive Rejection Sampling (Gilks and Wild, 1996) is used to generate samples from each full conditional distribution without needing to calculate the normalizing constant.

⁵⁴ Suppose that the product of the prior and likelihood is equal to $K(\theta_{-m}) \times g(\theta_m, \theta_{-m})$, where θ_m is the m^{th} unknown parameter, θ_{-m} is the vector of the other $n-1$ unknown parameters, and $K(\theta_{-m})$ is an unknown normalizing constant that will depend upon the data values and the values of the remaining $n-1$ parameters. To obtain the full conditional distribution, $K(\theta_{-m})$ can, in principle, be calculated as the reciprocal of the integral of g over the range of possible values of θ_m , treating the other parameters as constants, but this calculation is often very difficult analytically. Adaptive rejection sampling (ARS; Gilks and Wild, 1992) randomly generates values of θ_m from g , without knowing the normalizing constant. The method requires that the function g is log-concave in θ_m , which holds for many distributions if the parameters are appropriately defined. The method may need to generate and reject several random values until the final value is accepted, but at each rejection, more exact bounds for g are calculated so that the probability of future rejections rapidly decreases. If the full conditional distribution is not log-concave, then the Metropolis-Hastings algorithm can instead be used to generate values from the full conditional distribution without knowing the normalizing constant.

distribution multiplied by the dose-response function, integrated over the parameter space. This can be thought of as the dose-response function averaged over the uncertainty distribution. The predictive Bayesian method has the advantage of producing an estimated dose-response function that is more protective of public health than the maximum likelihood estimate, because at low doses the estimated risk is generally higher. The method also has the advantage of avoiding the need to specify a frequentist confidence level or a standard Bayesian prediction interval probability level, which avoids potential inconsistencies when comparing risks from different health stressors; the more risky stressor can depend upon the probability level chosen. On the other hand, upper bounds of confidence or prediction intervals can be thought of as estimating the risk under a “worse-case” scenario, and regulators may prefer a worse-case scenario approach to the predictive Bayesian approach that represents the average scenario (averaging estimates over the parameter uncertainty).

G.6.1 Comparison of Bayesian and Frequentist Methods

Before Bayesian methods were applied to MRA, risk assessors were generally limited to simpler model formulations and approximate uncertainty estimates. Risk assessors also could not take advantage of any available subjective information on the values of the unknown parameters. An advantage of the Bayesian approach over the frequentist approach is the ability to incorporate prior information, although for the MRAs in the current literature this is not very important because the prior information is too limited and so non-informative priors have been used. A more important advantage is that the uncertainty intervals from a Bayesian analysis are easier to interpret and are usually not interpreted incorrectly—a Bayesian 95% credible interval for the dose-response is interpreted as having a 95% probability of including the true probability of illness (or infection) given the available data. The risk (probability of illness or infection) is treated as being random. A frequentist 95% confidence interval is properly interpreted as having a 95% probability of including the true probability of illness (or infection) in an identical future experiment, so that 95% of a large number of identical future experiments will give confidence intervals that include the true risk. The risk is treated as being an unknown constant. Lay persons will very often incorrectly interpret the confidence interval as if it had the same meaning as the Bayesian credible interval.

Bayesian dose-response uncertainty calculations using MCMC also have the advantages of being easier and more exact than the frequentist confidence intervals. Because the dose-response function is a complicated function of multiple parameters, the confidence intervals are hard to calculate or approximate analytically. The bootstrap or similar Monte Carlo resampling methods can avoid these difficult analytical calculations but this often requires more computation than MCMC. Furthermore, the large sample theory estimates of the confidence intervals are poor approximations for the small samples typically found in MRA. While bootstrap estimates are more reliable for small samples, they are also approximations to the true uncertainty distributions, even if the number of bootstrap simulations is tending to the infinite limit. The MCMC uncertainty estimates are exactly correct for the posterior distribution assuming that the burn-in period is sufficiently long that the chain can be considered stationary (ignoring the imperfect nature of computer random number generation).

A further major advantage of the Bayesian method for MRA is the ability to use a hierarchical Bayesian model to model cases where the host or pathogen response parameters vary over the population of humans or organisms (e.g., see Messner et al., 2001, discussed below). This type of meta-analysis is easier to apply in a Bayesian framework.

The major disadvantage of the Bayesian approach is the requirement for developing a prior distribution that, in principle, is subjective and thus depends on the information available to the investigator. Different investigators can choose different priors for the same model formulation, even if the prior are “non-informative.” The subjective nature of a prior distribution can be disturbing. On the other hand, Bayesian statisticians often point out that the investigator’s choice of dose-response function or other mathematical model is also a subjective choice.

G.6.2 Applications of Bayesian Methods to Microbial Risk Assessment

In one of the earliest Bayesian analyses of microbial risks, Teunis and Havelaar (2000) modeled rotavirus, *Campylobacter*, and *Vibrio cholerae* dose-response data using Poisson, beta-Poisson, and gamma-Poisson models. In the Poisson model, an individual is exposed to a number of organisms (cfu) that is assumed to have a Poisson distribution with a mean dose D equal to the volume ingested multiplied by the average number of cfu per unit volume. Each single organism independently has the same hit probability (r) of infecting the subject. It follows that the probability of infection at dose D is exponential with parameter $r \leq 1$:

$$\text{Prob (infected} \mid D, r) = 1 - e^{-rD}. \quad [\text{G-16}]$$

In the beta-Poisson model, r is assumed to vary among hosts or organisms with a beta distribution with parameters $\alpha > 0$ and $\beta > 0$. This gives the dose-response function as follows:

$$\text{Prob (infected} \mid D, \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -D). \quad [\text{G-17}]$$

The function ${}_1F_1$ is the Kummer confluent hypergeometric function.

The gamma-Poisson model is an approximation to the beta-Poisson model of the following form:

$$\text{Prob (infected} \mid D, \alpha, \beta) = 1 - (1 + D / \beta)^{-\alpha}. \quad [\text{G-18}]$$

This model is also obtained if either r has a gamma distribution that includes all values $r > 0$ (even though the hit probability r cannot exceed 1), or, more realistically, if the concentration has a gamma distribution. The model in Equation 18 was originally called the beta-Poisson model, because it was derived as an approximation to the exact Beta-Poisson model in Equation 17. The same three equations can be used to model the probability of illness, particularly for outbreaks or studies, where the numbers of infection cases are not reported.

Teunis and Havelaar (2000) fitted all three models and estimated the parameters r , α , and β using maximum likelihood (i.e., choosing values to maximize the probability of the data given the parameters, which is a product of binomial probabilities). The likelihood is given by Equation 1 above. Using the maximum likelihood estimates of the parameters in the dose-response function

(Equations 1, 2, or 3) provides the maximum likelihood estimate of the dose-response function. They also estimated approximate 95% confidence intervals and regions for the parameters using the likelihood.

The authors also used a bootstrap resampling method to estimate the uncertainty of the dose-response function by randomly sampling with replacement from the original data and fitting the dose-response model to each of the resampled data sets. Thus, each bootstrap sample gives a different dose-response function. For each dose D , a 95% confidence interval for the probability of being infected is given by the 2.5th to 97.5th percentiles of the set of bootstrap dose-response functions evaluated at dose D .

Teunis and Havelaar (2000) used a Bayesian MCMC approach primarily to more easily compute the uncertainty estimates and to compare their maximum likelihood estimates with Bayesian estimates. Their prior distribution for the parameters α and β assumes they are independent and that their logarithms (base 10) have a uniform distribution from -12 to +6. The MCMC method was used to generate pairs of parameter values α and β from the posterior distribution. They found that the likelihood-based confidence regions for the parameters probability matched well to the sampled Bayesian posterior distribution. For each parameter pair, the dose-response function was calculated. For each dose D , a 95% credible interval for the probability of being infected is given by the 2.5th to 97.5th percentiles of the set of dose-response functions evaluated at dose D .

Messner et al. (2001) used Bayesian methods to analyze the results of three human volunteer studies, each using different isolates of *Cryptosporidium*—IOWA, TAMU, and UCP. For each individual study, they fitted an exponential model (Equation 2) by maximum likelihood and then computed the maximum likelihood estimate of the dose-response function. They compared their results with a Bayesian analysis based on the assumption that $\log(r)$ has a uniform distribution over the entire real line.⁵⁵ The means and medians of the Bayes predictive distributions were very similar to the maximum likelihood estimates. Given the assumed distribution for $\log(r)$, its posterior density is proportional to the likelihood function.

To combine results from the three studies in a meta-analysis, Messner et al. (2001) used a hierarchical Bayes model that had several groups of parameters. At the first level, the hyperparameters are parameters with a prior distribution that does not depend on any other parameter. At the second level, some parameters are assigned distributions that depend upon the values of the hyperparameters. At the third level there are parameters that have distributions that depend upon the first and/or second level parameters. The hierarchy can have multiple levels, although most applications to MRA have at most two levels.

Messner et al. (2001) defined two hyperparameters μ and σ . Their prior distributions were not listed in their paper. The parameters r for each study were assumed to be independently drawn from a normal distribution with mean μ and standard deviation σ . This normal distribution represents variability of r between isolates, i.e., the probability of infection from a dose of a

⁵⁵ Such a prior is not a proper probability distribution because it cannot integrate to 1, but in many cases an improper prior can be used to calculate a valid posterior distribution. This improper uniform prior can be regarded as being the limit of a uniform distribution for $\log(r)$ over the range $-M, M$ as M tends to infinity.

single organism depends upon the isolate. Thus, the model has a total of five parameters. The MCMC method using the Gibbs sampler was used to generate samples of parameter vectors from the posterior distribution given the data from all three studies. Eighty percent credible intervals (10th to 90th percentile of the posterior distribution) were thus calculated for the parameters and for the dose-response function at a dose of one oocyst.

Teunis et al. (2004) used Bayesian modeling to analyze data from an outbreak of e-coli O157:H7. This study included both illness and infection counts. They modeled the dose-response functions using the beta-Poisson model shown in Equation 17. A non-informative prior was selected such that $u = \alpha/(\alpha + \beta)$ is uniform from 0 to 1 and $v = \log_{10}(\alpha + \beta)$ is normally distributed with mean zero and standard deviation 10. The transformed parameter u is the mean of the beta distribution for r , and v is inversely related to the variance of the beta distribution. The parameters u and v are assumed independent. The transformation improves the parameter estimation since if there is only a single dose value, α and β are highly correlated. The parameter values corresponding to the mode of the posterior distribution were calculated directly by numerically maximizing the posterior probability, which is the same as maximizing the product of the prior distribution and the likelihood. The posterior probability equals the prior multiplied by the likelihood and divided by the normalizing constant, which does not depend upon α and β . Using the posterior mode parameter values in Equation 17 gives the posterior mode dose-response equation. The uncertainty of this dose-response function was characterized using MCMC sampling of parameter vectors. The dose-response function was calculated for each parameter vector and the percentiles of the response probability for each dose were plotted. Frequentist likelihood ratio tests were used to compare different dose-response models.

Teunis et al. (2005) analyzed *Campylobacter jejuni* dose-response using Bayesian methods. Data from both a human volunteer study and an outbreak caused by drinking raw milk were combined in this analysis. The model incorporated both the probability of infection and the conditional probability of illness given infection. First, for the outbreak a certain probability of illness (p_0) was assumed for those who were unexposed to the raw milk but might have become ill due to an alternative route of transmission. Second, a beta-Poisson model was used to model the probability of infection given a mean dose (D). Third, a model for the conditional probability of illness, given that the individual is infected and had mean dose D , was developed as follows:

$$\text{Prob}(\text{ill} \mid \text{infected}, D, r, \eta) = 1 - (1 + \eta D)^{-r}. \quad [\text{G-19}]$$

Non-informative prior distributions for the parameters were defined by assuming that all parameters are independent and that $\text{logit}(\alpha/(\alpha + \beta))$, $\log_{10}(\alpha + \beta)$, $\log_{10}(r\eta)$, $\log_{10}(r/\eta)$, and $\text{logit}(p_0)$ are all normally distributed with mean 0 and standard deviation 10. By definition $\text{logit}(x) = \log(x/(1-x))$. The posterior mode parameter values were calculated by directly maximizing the posterior probability. These values were used to compute the posterior mode dose-response functions for the probability of infection (Equation 17) and the probability of illness given infection (Equation 19). Uncertainty intervals for these dose-response functions were computed by using MCMC to simulate vectors of parameter values.

Teunis et al. (2008a) analyzed data from eight outbreaks of *E. coli* O157:H7 using a hierarchical Bayes model. A homogeneous exposure model used a beta-Poisson dose-response function for

the probability of illness (Equation 17). A heterogeneous version of the exposure model also included known values of a dispersion parameter, treated as being the shape parameter for a gamma distribution of the microbial concentrations. Using a different notation to that used in the paper, the hyperparameters m_1 , m_2 , s_1 , and s_2 were assumed to be independent and have distributions such that m_1 and m_2 were normally distributed with mean -8 and standard deviation 8, and s_1 and s_2 were gamma (0.001,1000) distributed. For outbreak i , $\log_{10}(\alpha(i)/(\alpha(i)+\beta(i)))$ and $\log_{10}(\alpha(i)+\beta(i))$ were assumed independently normally distributed with means m_1 and m_2 and standard deviations s_1 and s_2 . To obtain an overall group dose-response function, representing the dose-response function for a future random outbreak, the α and β parameters of that outbreak were assumed to be generated from the prior distribution of the hyperparameters; that is, $\log_{10}(\alpha/(\alpha+\beta))$ and $\log_{10}(\alpha+\beta)$ were assumed to be independently normally distributed with means m_1 and m_2 and standard deviations s_1 and s_2 .

Teunis et al. (2008a) fitted these models using MCMC. The posterior mode dose-response function for each outbreak was estimated by finding the sample parameter vector with the highest value of the joint posterior (partial) probability, which is the product of the prior density for the hyperparameters, the conditional density for $\alpha(i)$ and $\beta(i)$ given the hyperparameters, and the likelihood for the outbreak i . The overall estimates of the dose-response function for a future outbreak were estimated by sampling α and β from the prior distribution of the hyperparameters and computing the dose-response function for each pair. This gives a set of dose-response functions. For each dose, the percentiles of the probability of being ill were computed and plotted as a contour plot.

Teunis et al. (2008b) used Bayesian methods to analyze dose-response functions for the Norwalk virus based on a volunteer study. Similar methods to the above studies were employed so the details are not discussed here.

Englehardt (2004) compared maximum likelihood methods to a predictive Bayesian dose-response approach. The method was applied to rotavirus data. First, he discussed the likelihood-based Benchmark Dose Method, which computes a confidence interval for the dose at which a certain change or percentage change in risk occurs and defines the benchmark dose as the lower confidence value. He pointed out that for two different health stressors, it is possible that the dose-response curves can intersect, in which case the more risky stressor (the stressor with the least benchmark dose) depends upon the confidence level chosen. A similar issue arises with Bayesian analyses using credible intervals to account for uncertainty; that is, the results depend upon the assumed “confidence” level. Englehardt recommends averaging the dose-response function over the posterior distribution of the parameters. In other words, the predictive Bayesian dose-response model for dose D is calculated as the integral of the posterior distribution of the parameters given the data multiplied by the dose-response function at dose D . This integral is over the entire probability space. The predictive Bayesian dose-response is the unconditional dose-response function and can be thought of as the dose-response function averaged over the uncertainty distribution. This can be compared to frequentist or more standard Bayesian approaches for which upper bounds of confidence or prediction intervals can be thought of as estimating the risk under a “worse-case” scenario. Regulators may prefer a worse-case scenario approach to the predictive Bayes approach, which represents the average scenario.

Englehardt (2004) applied the predictive Bayes approach to rotavirus data using the beta-Poisson model (Equation 3). The maximum risk for any dose D is calculated using the exponential model (Equation 2) with $r = 1$, which assumes a hit probability equal to 1 so that infection is guaranteed (100%) if any organisms are ingested. The minimum risk for any dose D is assumed to be obtained from the maximum likelihood estimates of the parameters α and β . The predictive Bayes dose-response function has a risk between the minimum and maximum risk. Englehardt (2004) points out that in general, if enough data are available, then at low doses, the predictive Bayes risk will be lower than the observed risk (proportion of illnesses), so that the approach is conservative (health-protective) compared to maximum likelihood methods. To calculate the posterior distribution, Englehardt used the MCMC method assuming an improper uniform prior for α and β . It is not clear from the paper how the integral of the posterior multiplied by the dose-response function was calculated. Although direct numerical integration is possible, in principle, a reasonable approach would be to compute the dose-response function (probability of illness) for each dose D and each sampled pair of parameter values, and then average the probability of illness over the entire sample. Note that Englehardt defines the normalizing constant k as the constant that normalizes the likelihood function. This is not correct in general since the normalizing constant k normalizes the posterior distribution (i.e., the product of the prior and the likelihood).

Englehardt and Swartout (2004) applied the predictive Bayes approach to the *Cryptosporidium parvum* data analyzed by Messner et al. (2001). However, these analyses separated out the results for subjects with Ab+ and Ab- serum-antibody status. First, maximum likelihood estimates for the beta-Poisson models were computed for each study (isolate) and Ab+ or Ab- status. Second, a representative population of sensitive, Ab+, and Ab- subjects was simulated using the maximum likelihood fitted dose-response functions; sensitive subjects were assumed to always respond at the doses tested. Each simulated population is a parametric bootstrap sample. A beta-Poisson model was fitted to each bootstrap sample using maximum likelihood. The set of maximum likelihood estimates was used to compute 95% confidence intervals for the probability of infection for each strain. The hit probability, r , for each isolate was estimated as the mean over all the bootstrap distributions for that isolate.

Englehardt and Swartout (2004) computed a predictive Bayes distribution for the r for a random isolate and for the dose-response function. For a random isolate, r is assumed to have a beta distribution with parameters α and β , assigned a joint uniform prior. The likelihood of the three observed r values is given by the product of three beta distributions; each observed r value is the mean for the bootstrap simulations of that isolate. Thus, the predictive Bayes distribution for r is defined by multiplying the beta distribution for r by the posterior probability for α and β given the three sampled values of r , and then integrating over the parameter space. This is the marginal distribution of r . To obtain the predictive Bayes dose-response function, the marginal probability for r was multiplied by the exponential dose-response function (Equation 2) and integrated over $0 \leq r \leq 1$.