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MICROBIAL RISK ASSESSMENT GUIDELINE

PATHOGENIC MICROORGANISMS WITH FOCUS ON FOOD AND WATER

Prepared by the Interagency Microbiological Risk Assessment Guideline Workgroup

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PREFACE

This Microbial Risk Assessment (MRA) guideline is written by microbial risk assessors at the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) and the U.S. Environmental Protection Agency (EPA). It serves as a resource for these agencies, their agents, contractors, and stakeholders. Other Federal agencies expressed interest in the development of this guideline and provided experts to participate in this interagency effort (see list of participants). The working group followed the Office of Management and Budget's (OMB's) *Good Guidance Practices* while developing this guideline (OMB, 2007a).

In recognition of the needs and mandates of the participating agencies and the various statutory authorities that may apply to MRA, this guideline emphasizes the need for a flexible framework for conducting microbial risk assessment. It provides general, broad fundamental risk assessment principles specifically for microbial risks, but as a guideline it is not prescriptive nor does it supplant the internal practices or policies of any Federal agency. Users have the flexibility to adapt pertinent sections to relevant statutory authorities and purposes if needed. The intended audience is individuals with some knowledge of microbiology and basic understanding of risk assessment principles but some basics may be presented at the introduction to a topic. This guideline can be periodically updated, particularly as more information becomes available.

The severity and duration of illness caused by exposure to pathogens vary considerably. Many human pathogens found in food, water, and the environment cause acute diseases that have short incubation periods, symptoms typically lasting several days to a week, and usually non-lethal, common gastrointestinal effects but with complete recovery from the illness. However, some pathogens associated with the gastrointestinal tract may cause more serious diseases or sequelae, such as reactive arthritis, cancer, Guillain-Barré syndrome, and juvenile-onset diabetes that may have long-term implications. Further, there are indigenous water-based pathogens, such as *Legionella* spp. and *Mycobacterium* spp., that can grow in biofilms and their inhalation via aerosols may cause pneumonia. Some disease manifestations can be fatal. Applying risk assessment approaches associated with MRA procedures discussed in this guideline help risk assessors characterize the common exposure sources, causative agents, associated symptoms, contributing immunity factors, and other common threads contributing to chronic illness. This guideline is for human health MRAs, and does not include MRAs conducted for ecological protection (e.g., wildlife, habitats).

This guideline does not specifically address scenarios related to biological warfare agents, airborne microbial hazards, or agriculturally or industrially important microorganisms, oligonucleotides, prions, preformed microbial toxins, and other submicrobial entities. These agents have many unknowns associated with their sources, modes of "infection" and disease, transmissibility, and survivability. Nonetheless, information in this guideline may provide information to risk assessors addressing these issues.

ABBREVIATIONS

ACSSuT	Ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline
AFLP	Amplified fragment length polymorphism
AIPH	Army Institute of Public Health (formerly U.S. Army Center for Health Promotion and Preventive Medicine [USA CHPPM])
ALOP	Appropriate level of protection
AOAC	Association of Analytical Communities
APHIS	Animal and Plant Health Inspection Service (U.S. Department of Agriculture)
ASM	American Society for Microbiology
Bcc	*Burkholderia cepacia* complex
BSE	Bovine Spongiform Encephalopathy
°C	degrees Celsius
CART	Classification and regression tree
CARVER	Criticality, Accessibility, Recuperability, Vulnerability, Effect, and Recognizability
CDC	Centers for Disease Control and Prevention
CEA	cost-effectiveness analyses
CFSAN	Center for Food Safety and Applied Nutrition
CFR	Code of Federal Regulations
cfu	colony forming unit
CWA	Clean Water Act
DALYs	Disability-adjusted life years
DHS	Department of Homeland Security
DNA	Deoxyribonucleic Acid
DOD	Department of Defense
DT104	Definitive type 104 (p48)
ECSSC	European Commission Scientific Steering Committee
EPA	Environmental Protection Agency
°F	degrees Fahrenheit
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FIRRM	Foodborne Illness Risk Ranking Model
FSIS	Food Safety and Inspection Service
GI	gastrointestinal
GRAS	generally recognized as safe
gyrB	DNA gyrase, subunit B
HACCP	Hazard Analysis Critical Control Point
HC	Hazard Characterization
HI	Hazard Identification
HIV	Human immunodeficiency virus
HYEs	Healthy-years equivalents
ID_{50}	Median Infectious dose
ILSI	International Life Science Institute
LD_{50}	Median lethal dose

LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
MAC	*Mycobacterium avium*-Complex
MCMC	Markov Chain Monte Carlo Simulation
MILYs	Morbidity Inclusive Life Years
MLGT	Multilocus genotype sequencing
MLST	Multilocus sequence typing
MRA	microbial risk assessment
MRM	microbial risk management
NASA	National Aeronautics and Space Administration
NCEZID	National Center for Emerging and Zoonotic Infectious Diseases
NCRP	National Committee on Radiation Programs
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health (Centers for Disease Control)
NOAEL	No observable adverse effect level
NRC	National Research Council
OECD	Organization for Economic Cooperation and Development
OMB	Office of Management and Budget
OPP	Office of Pesticide Programs (US Environmental Protection Agency)
OPPT	Office of Pollution Prevention and Toxics (US Environmental Protection Agency)
ORD	Office of Research and Development (US Environmental Protection Agency)
OSA	Office of the Science Advisor (US Environmental Protection Agency)
OW	Office of Water (US Environmental Protection Agency)
P/CC	Presidential/Congressional Commission
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
PFGE	Pulsed field gel electrophoresis
pfu	plaque forming units
QALYs	Quality-adjusted life years
RAF	Risk Assessment Forum (US Environmental Protection Agency)
RAPD	Random amplification of polymorphic DNA
rDNA	Ribosomal deoxyribonucleic acid
REP-PCR	Repetitive element polymerase chain reaction
R&D	Research and development
SARS	Severe Acute Respiratory Syndrome
SDWA	Safe Drinking Water Act
SNP	Single nucleotide polymorphism
TAMU	Texas A&M University
TB	Tuberculosis
TCCR	Transparency, Clarity, Consistency, and Reasonableness
U.S.	United States
USDA	U.S. Department of Agriculture
VBNC	Viable-but-not-culturable
vCJD	variant Creutzfeldt-Jacob Disease

VOI	Value of information
WHO	World Health Organization

EXECUTIVE SUMMARY

Modern societies have learned to reduce the impact of disease-causing microorganisms (pathogens) by adopting various sanitary control measures, such as farm-to-fork processes in food production and treatment plants for drinking water and wastewater. Nonetheless, our aging and more vulnerable population groups combined with the emergence of drug resistant pathogens and enhanced global spread of human pathogens provides a breeding ground for novel and reemerging diseases. This microbial risk assessment guidance document is written for risk assessors from participating federal agencies to improve the quality and consistency of microbial risk assessments. The guidance takes on a question/answer format for these practitioners to make it more approachable by a wide audience.

While some federal agencies have an established record of conducting and advancing chemical risk assessments (e.g. National Research Council reports, 2007, 2009), microbial risk assessment has not received as much attention or support. There are several possible reasons for this but a significant one may be due to the challenges posed by microbial risk assessments that are not considered in classical chemical risk assessments. These challenges include the immune status of the host organism, person-to-person transmission, and re-growth of pathogens both in the environment and in the host. Further, no guideline has been previously developed by U.S. agencies addressing the full process of microbial risk assessment. As a consequence, various approaches have been used nationally, some based on the International Life Sciences Institute framework for microbial risk assessment (ILSI, 1996, 2000).

The *Microbial Risk Assessment Guideline: Pathogenic Microorganisms with Focus on Food and Water* addresses the entire risk assessment process from an introduction to terminology and roles of the participants to planning the risk assessment, identifying and characterizing the hazard, assessing how the size of an outbreak may be affected by the dose (exposure assessment) or how the severity of the disease may be affected by the pathogen and its response within the human host (dose-response assessment). The document describes the importance of addressing the routes of exposure, transport media, uncertainties, and assumptions for exposure and the other components of the risk assessment paradigm when characterizing risk, and also provides information about microbial risk management and risk communication. The goal of this document is to produce a more harmonized treatment of microbial risk assessment across participating federal agencies.

The Introduction (Chapter 1) lays out the purpose of the guideline, describing some of the relevant history of the guideline and noting recommendations to develop a microbial risk assessment guidance document based on the modified chemical risk assessment paradigm. Next, Planning and Scoping (Chapter 2) describes the importance of clearly identifying the purpose of the risk assessment at the outset. From the articulated purpose, one determines the resources needed, including expertise, assesses the current state of knowledge about the issue, and decides how to proceed with a clearly defined vision of what the decision-maker will need, ensuring that team members understand the goals of the assessment and that they agree on the approach. The level of

rigor applied during the planning and scoping phase of the risk assessment often has a significant bearing on the quality and utility of the final product.

In order to properly conduct the risk assessment, the causative agent(s) must be characterized (Chapter 3, Hazard Identification and Hazard Characterization). In the case of an outbreak of a disease, the team typically works backwards evaluating possible exposure pathways to the identified source of the pathogen(s). However, in many cases the assessors start with known agent(s) and anticipated source-to-receptor scenario(s) in an attempt to predict outcomes and to provide advice for risk management. In both cases, a qualitative characterization of the hazard(s) and likely consequences of exposures are identified with respect to potential human impact, including consideration of multiple life stages.

Environmental stressors, biological or otherwise, normally exhibit an increasingly pronounced effect with higher exposures, either in severity of the effect or fraction of the population affected. As the host is exposed to more pathogens, the potential for disease and/or the nature of the effect becomes more evident. This biological gradient is referred to as the dose-response relationship (Chapter 4), which for pathogens is generally based on the possibility, although very low likelihood, that even a single pathogen could cause infection. The likelihood of infection increases in a mathematically-modeled sigmoidal fashion with increases in pathogen dose. This simplified model of dose-response is called a “single-hit” model. A range of such models are described in Chapter 4 for the different groups of pathogens, based on human exposure data or animal models.

A critical component of any risk assessment is the exposure assessment. The nature of the risk is based on the level of exposure to the agent. From a management perspective, the frequent goal of a risk assessment is to reduce risk. The exposure assessment assesses the magnitude of exposure and hence the chance for the onset of disease, and can help identify means to reduce risk from a pathogen by reducing exposure. Chapter 5 provides guidance for conducting an exposure assessment for prospective and retrospective assessments when exposure is through water and food media. Related topics include the measuring and modeling of exposure data, and how to report variability and uncertainty with data.

Chapter 6 covers the integration of the hazard identification and hazard characterization, dose-response information, and exposure data into a risk characterization. The risk characterization is designed to present the output of the information into a form that addresses the issues and concerns raised during planning and scoping, and meets the needs of the decision-maker. The risk characterization presents the potential for disease from exposure under a given scenario or it helps to identify areas that can be modified to reduce the potential for a disease outbreak. A good characterization of the risk from an anticipated pathogen exposure reports the strengths and limitations of the assessment in a clear and concise manner, noting the assumptions, characterizing the quality of the data, and reporting uncertainties. The risk characterization informs the decision-maker and serves as the basis for the risk communication content.

The two final chapters cover risk management and communication. Risk management (Chapter 7) describes the role of the risk manager and provides information about applying the risk characterization to management decision-making. Risk management involves the steps that a risk manager may take to reduce risks. Effective risk communication (Chapter 8) ensures that the communication and outreach efforts associated with the microbial risk assessment are appropriately planned and that the results are accurately and appropriately communicated to the decision-maker and stakeholders.

This *Microbial Risk Assessment Guideline* provides valuable tools and information for risk assessors on the steps and components involved in microbial risk assessment. By presenting all of the components for a microbial risk assessment in a single document, the individual components are linked together in a framework that is easy to follow and use. Government programs that adopt this guideline for microbial risk assessment are expected to produce more consistent and transparent risk assessment documents containing a more complete complement of information used by decision-makers.

1. INTRODUCTION

A microbial risk assessment (MRA) is a valuable tool for organizations tasked with understanding, reducing, and preventing risks presented by hazardous microorganisms, whether natural or anthropogenic, intentional or unintended. Increasing globalization has compounded these risks, with the broadening and often rapid distribution of illnesses. Clear and credible risk assessment methods are proving ever more necessary for agencies to address both current and future risks associated with contamination of air, water, soil, and food by bacteria, fungi, protozoa, viruses, and their toxins.

This guideline is intended to lay out an overarching approach to conducting MRAs and to introduce the users to tools and methods needed to do them. Additionally, it will promote consistency and improve transparency in the way MRAs are conducted. This document provides information to be used by risk assessors and decision makers when assessing the safety of water or food.

This guideline focuses primarily on infectious diseases associated with the gastrointestinal (GI) tract and fecal or oral transmission of the causal agents mainly in food and water, but clearly has application to other scenarios, such as inhalation to microorganisms. Agencies that need to be concerned about pathogens often have similar requirements to protect the health of potentially exposed people. For example, a number of pathogens of concern originate in the GI tract of humans and animals, and can potentially contaminate food, surface water, or drinking water. The agencies that regulate food and environmental contaminants recognize that the ultimate sources of pathogens are the same no matter the affected media (e.g., water and food). Because the health effects and dose-response relationships for many of the pathogens are similar regardless of media, it is useful to have common principles and approaches to assess risks across media and exposure settings.

1.1 Who is this Guideline Written For?

The target audiences for this guideline are microbial risk assessors and related professionals, such as risk managers in agencies and the private sector, as well as citizens interested in microbial contamination of food and water. It is written for persons with some knowledge of microbiology and also a fundamental understanding of risk assessment principles, but some basics are presented throughout this guideline. Further, it provides key points to consider, as well as useful tools and methodologies for preparing a microbial risk assessment.

For clarity and ease of use, the format of this guideline is in a question and answer format. The question poses an approach taken by the risk assessor asking a specific question (the use of “I” in many instances). The answer is a response to the assessor’s question (the use of “you” refers back to the risk assessor).

1.2 What are the Benefits of This Guideline?

Government agencies have conducted formal risk assessments for chemicals in food, water, and the natural environment for decades. These assessments originated in support of or in response to a number of laws and regulations directing federal agencies to control chemical contaminants in food and environmental media. In 1983, the National Research Council (NRC) of the National Academies published *Risk Assessment in the Federal Government; Managing the Process* (NRC, 1983; hereafter referred to as the "NRC 1983 report"). This document helped unify the risk assessment processes for chemicals in food and the environment and provided a framework that federal agencies, their clients, and the risk assessment community in general could apply in conducting risk assessments. Since then, virtually all U.S. regulatory agencies have cited the NRC 1983 report as providing essential guidance in conducting risk assessments.

Though the standard chemical risk assessment approach was established in the 1980s, a similar MRA approach could not be developed then due to a lack of essential information. At that time, these limitations included a lack of data, tools, and methods, such as comprehensive dose-response models, poor quantification of microbial occurrence, limited analytical methods (i.e., sensitivity, specificity, precision, and accuracy), and poor understanding of human immunological responses. Since the 1990s, the use of MRAs has gained greater credibility in the federal regulatory community as new information on the identification and occurrence of infectious microbial pathogens, the potential for human exposure, dose response, and attributable health effects became increasingly available. A number of mathematical models, protocols, and other tools have become available that allow MRAs to be conducted even with substantial variability, uncertainty, and lack of specific data; further, methods are now available to characterize such variability and uncertainty associated with data used in MRAs. During the 1990s, it also became apparent that the NRC 1983 report had some shortcomings for conducting MRAs because chemicals are different from microorganisms in a number of ways (see section 1.3). While agencies conducting MRAs have continued to rely on the NRC 1983 report generally, they have individually made adjustments to adapt it for MRA. For example, the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) used the international Codex Alimentarius Commission (Codex) framework, which follows the same overall structure as the NRC 1983 report. This document provided guidance for conducting an MRA of *Listeria monocytogenes* in ready-to-eat foods (FAO/WHO, 2004). The U.S. Environmental Protection Agency (EPA) used the framework in the NRC 1983 report to evaluate the public health impact of drinking water regulations for *Cryptosporidium* oocysts (EPA, 2006a).

The primary reason for this guideline is to provide risk assessors with a structured approach for microbial risk assessment. This guideline is more comprehensive than earlier guidance, such as the food safety/MRA frameworks that precede it (Codex, 1999; ILSI, 2000; FAO/WHO, 2006; Codex, 2007a, 2007b). The 2009 EPA Office of Water's *Protocol for Microbial Risk Assessment* provides some detail, but it is of course focused

on water issues (EPA, 2009a). That said, that publication and the ones identified above were useful for the development of this guideline.

1.3 What are Some Fundamental Differences between Microbes and Chemicals?

While chemical risk assessments and MRAs are conceptually similar, there are enough differences between chemicals and microorganisms (Ahl et al., 2003) that having an approach specifically covering unique microbial considerations is essential (i.e., a microbial risk assessment guideline). Even though the uniqueness of each chemical is considered individually in chemical risk assessment, some significant differences in MRAs are:

a) **Microbial growth and death** – Pathogens increase and decrease in number in the environment and in hosts. Different species, and even different strains within a single species, grow and die in unique patterns. In contrast, while chemicals can bioaccumulate, bioconcentrate, remobilize, and undergo transformations, they do not multiply in the environment or hosts. Both chemicals and pathogens can decrease; chemicals can be transformed or degraded, and pathogens can die or become unculturable but may remain infectious. In addition, environmental stresses can impact the virulence of some pathogens. In addition, microbial toxins can remain after the organism dies, and some enterotoxins are heat stable and resistant to degradation. These toxins cause many of the symptoms of GI illness.

b) **Host immunity and susceptibility** – Although body weight, age, and metabolic capacity differences are considered in chemical risk assessments, genetic and acquired differences in susceptibility are not considered in chemical risk assessments in the same manner as in MRAs. Chemical risk assessments use uncertainty factors derived from data on known sensitive populations to account for these host differences. MRAs use a dynamic model to determine immune status. Chemical risk assessments may consider the immune system if a chemical causes a hypersensitivity reaction. An infection resulting in illness due to a pathogen is, in some cases, highly dependent on the immune status of the individual, which can fluctuate based on the time since last exposure to the pathogen, presence of concurrent infections, and a number of other factors (e.g., life stage, nutrition, genetics). Other factors that can influence susceptibility but not necessarily through changes in immunity include concomitant illnesses and medications.

c) **Diversity of health endpoints** – The same dose of a pathogen may result in a broad range of health outcomes or endpoints depending on the characteristics of the host and the exposure scenario. Endpoints for the same dose could include asymptomatic infection, intensity ranging from mild to severe symptoms, different tissues or organs affected, acute symptoms, chronic symptoms, or death. Because susceptibility and immunity fluctuate in a population over time, the percentage of potential hosts that will experience the

different endpoints also fluctuates over time. Examples of different health endpoints for the same organism include: enterovirus infection can be asymptomatic or severe and cause diarrhea or viral meningitis. Infections in people with *Campylobacter* can be asymptomatic or mild, and also be acute or have chronic effects, such as arthritis, inflammatory bowel disease, or Guillain-Barré syndrome paralysis.

d) **Genetic diversity and evolution of microbial strains** – Microorganisms are genetically diverse, and allelic ratios (variations of the same gene) in a population can change significantly within a few generations. In addition, microbial genomes can evolve quickly (within days or weeks) through mutation or vertical gene transfer (within a species) or horizontal gene transfer (between different species, families, and higher taxonomic differences). Strains of the same species (e.g., *Cryptosporidium parvum*, *Escherichia coli*) can have multiple genotypes, potentially with different virulence for human hosts. Some pathogens (e.g., *Helicobacter pylori*, many viruses) behave like "quasi-species," which are fluctuating populations of genetically distinct variants that can co-exist within a single host (Boerlijst et al., 1996; Covacci and Rappuoli, 1998). Microbes represent a "moving target," because the distribution of strains and virulence factors can fluctuate rapidly in a given medium.

e) **Potential for secondary transmission** – Microbial infections can be transmitted between individuals and from animal species to humans (referred to as zoonotic transmission). With the exception of the mother-fetus and nursing mother-infant relationships, chemicals in tissues of exposed individuals are not known to be transmitted to other individuals. Chemicals that are on an exposed individual's clothing or skin can be transferred to household and other inanimate objects (fomites), but that transfer generally results in dilution of the chemical. Conversely, pathogen secondary transmission can amplify the consequences of the pathogen. Some microbes can remain viable for days, weeks, or months on surfaces, which increases the potential for transmission. For some pathogens, humans can become asymptomatic chronic carriers and thus can infect others and contaminate food and water sources without displaying symptoms themselves for prolonged periods.

f) **Heterogeneous spatial and temporal distribution in the environment** – Pathogens are typically heterogeneously distributed in environmental matrices. Pathogen growth may lead to clustered distributions, and pathogens may clump together or may be embedded in or attached to organic and inorganic particulate debris, making traditional concentration determinations difficult to obtain. Although the concentration in pipe scale and biofilms is also a problem for chemical contaminants, some pathogens can grow and/or be protected in these specific environments. Also, many types of pathogens occur only episodically and typically can be found only during short-lived

disease outbreaks (i.e., epidemics) in a community. Seasonal and event-related (wet weather) spikes are common. The matrix, which includes all the components of the media (e.g., particles, pH, and others), can influence the spatial distribution of microorganisms.

g) **Single exposure health outcome** – Chemical risk assessments are conducted for acute exposures that cause immediate health outcomes and also for chronic exposures with long-term health effects. For chronic exposure to chemicals, the risk may be from daily exposure over a 70-year lifespan, whereas for pathogens the risk may be from a single exposure with health effects noticeable within days or weeks. Some pathogens may cause later sequelae, which are health outcomes that appear much later than the original symptoms. Some sequelae are chronic. Unlike the long-term exposures for chemicals, MRAs typically do not consider longer-term risks due to pathogen exposure. However, an MRA should consider available information on sequelae if it is available.

h) **Wide range of microbial response to interventions** – Many risk assessments address risks to human health associated with media that have been subjected to some sort of treatment, such as wastewater treatment or strict processing of foods. Microorganisms respond with wide variability to environmental and treatment factors. For example, response to drinking water treatment needs to be taken into account when comparing microbial levels in ambient water and treated drinking water. In the Clean Water Act (CWA) 304(a) ambient water quality criteria, EPA made a policy assumption that drinking water treatment has no effect on chemical concentration when they determine what levels to set for ambient water (EPA, 2000b).[1]

i) **Detection method sensitivity** – While there are laboratory detection methods for many commonly found pathogens in food and water, microbial detection methods are not always sensitive enough to detect pathogens at a level of regulatory concern. This is not necessarily the case for all pathogens or all media, but does apply to some combinations of organisms and media. 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 1000 liters of water) are not sufficiently developed to be reliable. In short, the human body is a more sensitive detector of pathogens than many laboratory methods. In addition, the viable-but-not-culturable (VBNC) state is not detectable by traditional culture-based laboratory methods (see section 3.8)

j) **Population, community, and ecosystem-level dynamics** – Microbial pathogens have complex interactions with other members of their species, other species, and the abiotic environment. For example, pathogens compete with non-pathogens for resources, and many non-viral human pathogens have

[1] Except for disinfection byproducts

animal hosts that can greatly complicate the ecological dynamics of pathogen occurrence and distribution. For some pathogens, population dynamics are better characterized than for other pathogens, so information may be available or not.

k) **Routes of exposure** – Many routes of exposure are similar for chemicals and microorganisms; however, there are some potentially important differences. Dermal exposure may be important for some chemicals. The dermal exposure route is not necessarily important with microbial exposure because unbroken skin is a natural barrier for entry. However, dermal exposure to microorganisms can cause infections through broken or otherwise damaged skin. In addition, dermal contamination with pathogens can lead to oral exposure via transfer, from the hands for example, to consumed food or water. Other aspects may include consideration of direct person-to-person or person-to-environment-to-person routes. Transmission of some organisms may occur via one route of exposure and then transmitted to secondary hosts via a different route, such as oral ingestion of a virus leading to spread by respiratory droplets.

Many and sometimes all of these factors can be significant considerations in preparing an individual MRA, but the approach for dealing with each one may well be different in particular risk assessment scenarios.

1.4 What are the Components of an MRA that I Should Consider?

Risk assessment is widely recognized as a systematic way to prepare, organize, and analyze information to help make regulatory decisions, establish programs, and prioritize research and development efforts. The Codex Alimentarius Commission, established by FAO and WHO and recognized by the World Trade Organization as the relevant organization for international food safety standards and guidelines, defined risk assessment as "a scientifically based process consisting of the following steps (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization" in *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (Codex, 1999; hereafter referred to as the Codex framework). This is the basic framework elaborated in this guideline; it is in essence similar to the treatment in the NRC 1983 report.

The risk assessment process is used to facilitate the application of science to policy decisions. Risk assessment informs the risk management decision-making process and risk communication through organized scientific analyses of data related to a specified hazard. Risk assessment also can evaluate potential or proposed risk management strategies' impact on public health. Essentially, an MRA is the formal, scientifically based process to estimate the likelihood (probability) of exposure to a microbial hazard and the resulting public health (and/or environmental) impact from this exposure. Risk assessment not only includes the likelihood of exposure and the impact of that exposure, but also steps for planning, scoping, and hazard identification and

characterization. This guideline only focuses on MRAs conducted for public health purposes.

The intent of this guideline is to provide information for the components necessary for successfully conducting a risk assessment, quantitative or qualitative, including (Figure 1.1):

a) Planning and scoping, including problem formulation,

b) Hazard identification (HI),

c) Hazard characterization (HC),

d) Dose-response assessment,

e) Exposure assessment, and

f) Risk characterization.

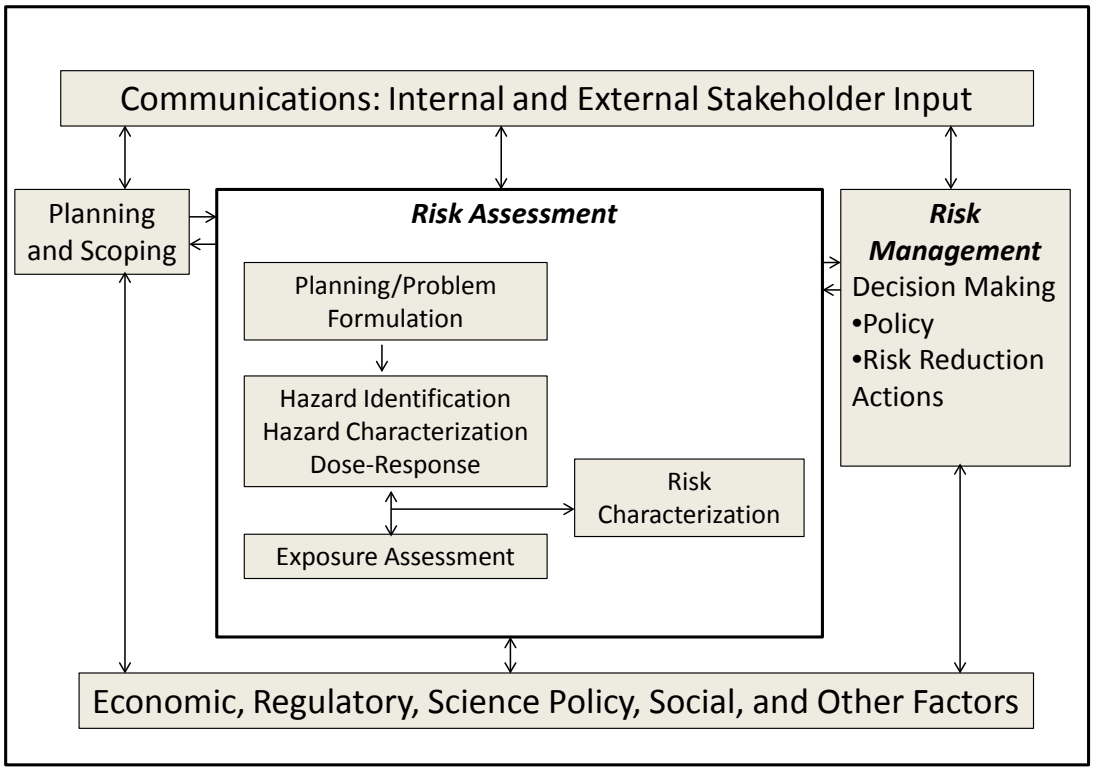

Figure 1.1 Risk Assessment Framework and its Relationship with Other Components of Risk Analysis (e.g., Risk Management, Risk Communication) (Adapted from NRC, 2009)

This guideline elaborates on these components that follow the Codex risk assessment framework. However, the chapter order reflects the discussion of the qualitative aspects of hazard characterization together with the qualitative aspects of hazard identification. The quantitative aspects of hazard characterization (i.e., dose response) are discussed in a separate chapter.

In addition to the components listed above, understanding the relationships and interactions between a microbial pathogen, its host, and the exposure to the pathogen in the environment is the key to determining the potential health impact a pathogen will have on an individual or population. The epidemiological triangle (disease triad) illustrates the inter-relationship between the host, pathogen, and environment components (Figure 1.2). For each of the components, several key factors should be considered. In many cases, a comprehensive quantitative treatment of all the factors to conduct an MRA is not possible because of data limitations; however, a qualitative consideration of the factors can be considered. Various points of the disease triad are discussed throughout this guideline.

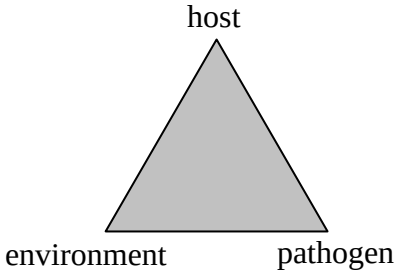

Figure 1.2 The Epidemiological Triangle (see e.g., Craun et al., 2006)

Finally, there is general recognition by risk assessors that an iterative approach is necessary when conducting an MRA to allow risk assessors to modify the risk assessment based upon changes in data availability and evolving agency policies (Presidential/Congressional Committee [P/CC], 1997). Often, the risk assessor must revisit the original charge or premise for conducting a risk assessment due to the lack of data, new data or interpretations, or uncertainty or variability in information. Revisions allow the risk assessor to incorporate new information, especially in areas found to be the most important to assessing risk. This approach ensures that the risk manager receives the most accurate interpretation of risks and makes the most appropriate management decisions. Risk assessors also can develop communication strategies in parallel with risk assessment iterations. The risk assessor should participate in the risk communication process as needed (see Chapter 8).

1.5 How is this MRA Guidance Related to Other MRA Frameworks/Guidelines that are Currently Available?

Two major sets of frameworks influenced the development of this guideline. The first set of frameworks includes the NRC 1983 report (NRC, 1983) and the NRC *Science and Decisions: Advancing Risk Assessment* (NRC, 2009). The NRC 2009 framework is an enhancement of the NRC 1983 report incorporating 25 years of risk assessment and regulatory experience. The NRC frameworks are geared to chemical risk assessments,

but have applicability to MRAs. They also are broadly applicable for many different exposure media. The second set of frameworks is the Codex *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (Codex, 1999) and Codex *Principles and Guidelines for the Conduct of Microbial Risk Management* (MRM) (Codex, 2007a). The Codex framework is specific to food media, but is tailored to microbial hazards. This guideline is broadly applicable to many media, but is tailored to microbial hazards to humans. Both sets of frameworks were important for the development of this guideline. These two sets of frameworks were not the only sources consulted for development of this guideline.

U.S. government agencies (e.g., EPA, USDA, FDA, DHS, and DOD), as well as government organizations from other countries (e.g., Canada, European Union, New Zealand), and international agencies (e.g., WHO, FAO, Codex, and the Organization for Economic Cooperation and Development [OECD] – for example, see FAO/WHO, 2003, 2006, 2008, 2009), have prepared various levels of guidance to support MRA applications. An EPA-sponsored study, *Foundations and Frameworks for Human Microbial Risk Assessment* (Parkin, 2008), presented an extensive search and evaluation of frameworks available for use in conducting MRAs. The study identified four general categories of frameworks applied in MRAs:

a) The 1983 NRC report;

b) A modified NRC 1983 approach without an explicit problem formulation (or planning and scoping) step;

c) A modified NRC 1983 approach with a problem formulation (or planning and scoping) step;

d) The International Life Science Institute (ILSI) approach (in association with the EPA's Office of Water) developed for water-based media (ILSI, 2000).

While most microbial risk assessors recognize shortcomings with a uniform, exclusive application of the NRC 1983 approach, most have not explicitly attempted to take a completely fresh look at approaches to conduct MRAs except for the ILSI (2000) approach which was loosely based upon EPA's draft *Ecological Risk Assessment Guidelines*.

EPA, FDA, USDA, DOD, and DHS have utilized their own unique approaches to conduct risk assessments, but it is important to keep track of other federally mandated requirements that may apply to MRAs. For example, when relevant, Executive Orders and OMB memorandums apply to MRAs (EPA, 2002b; OMB, 2007b; Presidential Memorandum, 2009; OSTP, 2010).

1.6 What MRA Principles Should I be Aware Of?

While there are differences between chemicals and microbes (as detailed in section 1.3), an MRA still adheres to the overarching principles for risk assessment in general. Many documents contain pertinent principles, three of which are highlighted here for MRAs (Codex, 2007b; EPA, 2000a; OMB, 2007b). Text Boxes 1.1, 1.2, and 1.3 provide a condensed version of the principles from these documents. For purposes of this guideline, the principles are condensed to a few major points:

a) MRAs should be "fit for purpose" to address the appropriate risk management problem(s)/issue(s).
b) MRAs should be as quantitative as possible. If quantitative data are not available, a qualitative approach can be used to address the current risk issue(s).
c) MRA assumptions and uncertainties need to be considered, explained, and documented.
d) Any MRA is developed using an iterative process, with each iteration increasing the quality of the data in order to reduce uncertainties and/or refocus the scope.

The Office of Science and Technology Policy (OSTP) directs that "When scientific or technological information is considered in policy decisions, the information should be subject to well established scientific processes, including peer review where appropriate, and each agency should appropriately and accurately reflect that information in complying with and applying relevant statutory standards" (Presidential Memorandum, 2009; OSTP, 2010).

By addressing these principles and adhering to well established scientific processes such as peer review, the correctness and the real world applicability of the MRA is most likely ensured.

Text Box 1.1 General Principles of MRA Adapted from Codex (2007b)

- Each risk assessment should be fit for its intended purpose.
- Each risk assessment should state the scope and purpose clearly.
- Experts involved in risk assessment should be objective and not be subject to any conflict of interest that may compromise the integrity of the assessment. Information on the identities of these experts, their individual expertise, and their professional experience should be publicly available, subject to national considerations. These experts should be selected in a transparent manner based on their expertise and their independence with regard to the interests involved, including disclosure of conflicts of interest in connection with risk assessment.
- Risk assessments should use available quantitative information to the greatest extent possible. It may also take into account qualitative information.
- Risk assessments should consider constraints, uncertainties, and assumptions that have an impact. Risk assessments should be based on realistic exposure scenarios. They should include consideration of susceptible and high-risk population groups.

- The risk assessment should be presented in a readily understandable and useful form.

Text Box 1.2 Principles of TCCR (Adapted from EPA, 2000a).

- **Transparency** shows that the methods and assumptions are clear and understandable (i.e., the use of methods, models, assumptions, and defaults are clear for others to correctly follow and understand the MRA).
- **Clarity** means the MRA is easy to understand and written in simple language.
- **Consistency** provides a context to compare to similar documents/assessments.
- **Reasonableness** explains founded and plausible professional judgments and assumptions.

Other factors are also important for the MRA, such as the assessment of data quality, data analysis, and peer review. Most agencies have addressed their needs for these in separate agency guidelines, and all have adopted OMB Information Quality guidelines relevant to these issues (OMB, 2002).

Text Box 1.3 Principles for Risk Analysis (adapted from OMB, 2007b)

- Agencies should employ the best reasonably obtainable scientific information to assess risks to health, safety, and the environment.
- Characterizations of risks and of changes in the nature or magnitude of risks should be both qualitative and quantitative, and consistent with available data.
- Judgments used in developing a risk assessment should state assumptions, defaults, and uncertainties explicitly.
- Risk assessments should encompass all appropriate hazards including attention to susceptible populations.
- Peer review of risk assessments can ensure that the highest professional standards are maintained.
- Agencies should strive to adopt consistent approaches to evaluating the risk.

1.7 How can the MRA be Used?

Although risk assessments conducted by different agencies are not used for the same purposes, agencies usually perform risk assessments with one or more of the following goals in mind (adapted from U.S. Army Center for Health Promotion and Preventive Medicine [USACHPPM], 2009). The goals could shape the planning and scoping discussion (see Chapter 2) and ultimately the risk assessment. The goals are:

a) To mitigate (e.g., adverse effects or risk from a specific event);

b) To confirm (e.g., to determine if regulations, policies, standards, criteria, and/or goals are adequate);

c) To decide whether and/or how to regulate (e.g., as needed to establish regulations, policies, standards, criteria, and/or goals);

d) To investigate (e.g., to determine research or other requirements that would enhance predictive and/or risk ranking capabilities, or facilitate completion of screening or feasibility assessments).

1.8 What Are Examples of Types of MRA?

Risk assessments can take various forms depending on the agencies' needs, risk management issue(s), or the immediate problem at hand.

a) **Screening** – Screening assessments often provide a conservative, health-protective estimate of possible risk that is based on the available data (this may be thought of as a "simple" quantitative risk assessment). Risk assessors often conduct screenings when a time critical decision is needed (e.g., quick mitigation action is required after an event; imminent exposure to a microbial hazard is identified). A risk assessor may resort to default assumptions to bridge data gaps that cannot wait for research to fill. In addition, screening assessments may actually provide the needed information that can address a risk management issue without having to initiate a more data or model intensive risk assessment.

b) **Risk ranking** – Risk ranking assessments compare the relative risk among several hazards. For example, this type of assessment might involve a single pathogen associated with multiple foods, a single food that has multiple pathogens, or multiple pathogens and multiple foods. Risk ranking assessments can help establish regulatory program priorities and identify critical research needs. The Food and Drug Administration/U.S. Department of Agriculture (FDA/USDA) *L. monocytogenes* risk assessment is an example of a risk ranking assessment (FDA/USDA/CDC, 2003).

c) **Product pathway analyses** – Product pathway assessments examine factors that influence the risk associated with specific vehicle/hazard pairs. For food, it ideally starts at the farm and ends with consumption. This type of assessment technique helps identify the key factors that affect exposure including the impact

of potential mitigation or intervention strategies on the predicted risk. The FDA *Vibrio parahaemolyticus* risk assessment is an example of a product pathway analysis (FDA, 2005).

d) **Risk-risk** – Risk-risk assessments consider a trade off of one risk for another (i.e., reducing the risk of one hazard increases the risk of another). As an example, an assessment could determine how treating drinking water with a chemical (risk to disinfection by-product exposure) would impact public health versus the impact of exposure to pathogenic organisms in water that is not treated.

e) **Geographic** – Geographic risk assessment examines the factors that either limit or allow the risk to occur in a given region. The assessment examines risk of introduction of disease agents through water, air, food animals, or animal products in the United States (e.g., intentionally as in a bioterrorism act or unintentionally). For example, the geographic approach might examine the risk of introduction of bovine spongiform encephalopathy (BSE) into the U.S. cattle herds and the subsequent risk of variant Creutzfeldt-Jacob Disease (vCJD) in humans by the transmission from cattle through meats and animal products.

f) **MRA within sustainability assessments** – Using a systems-level assessment over the lifetime of its technical components, sustainability assessments attempt to account for human health, ecosystem health, and economic considerations. The human health aspects include chemical and MRAs. This approach includes the MRA over the expected lifetime of the technical system and via all exposure pathways of pathogens to humans (e.g., drinking water, reuse waters, aerosols pathways, recreational exposures, and contaminated soils/foods).

g) **Threat and vulnerability assessments** – Threat and vulnerability assessments are specialized tools for evaluating the susceptibility of systems and facilities to potential threats, such as adversarial actions (e.g., vandalism, insider sabotage, or terrorist attack), natural disasters, and other emergencies. Although not strictly speaking a "risk assessment," the results can be similar where they identify threats and characterize the nature, probability, and magnitude of adverse effects, and the results can help to inform risk management decisions. For example, it may be necessary to assess the risks associated with intentional contamination of the food or water supply with biological agents or release of biological agents as an aerosol into highly populated indoor or outdoor public areas. These assessments can identify corrective actions that can reduce the risk or lessen the severity of potential consequences. The CARVER plus Shock method (FDA, 2007) is a preemptive targeting prioritization tool, which has been adapted for use in the food sector.

1.9 What Types of Decisions within Risk Assessment are Science Policy?

Science policies, sometimes codified in an agency's procedures, are used to aid both the assessor and decision maker in the decision-making process. Science-policy

positions and choices are by necessity utilized during the risk assessment process in two major ways. First, there are some basic, fundamental science-policy positions that frame the risk assessment process to ensure that the risk assessments are appropriate for a particular decision. These scoping "boundaries" for the risk assessment are articulated during the planning and scoping process and ultimately explained clearly in the risk characterization (i.e., what will be addressed in the risk assessment for decision purposes, but also just as importantly, what will not be addressed and why [e.g., not pertinent to the decision needed]). These science-policy positions not only shape the risk assessment process, but are usually factors in the risk management process outside the risk assessment.

Second, the use of default assumptions in a risk assessment is a science-policy choice often invoked when there is a lack of data. These choices are more specific than the framing science policies mentioned above. Given the nature of uncertainty and data gaps, default assumptions (sometimes simply called defaults) address these uncertainties when data are unavailable or otherwise not suitable for use. A default assumption is the best option available in the absence of data to the contrary (NRC, 1983). The NRC supports the use of default assumptions in its review of risk assessment practices in *Science and Judgment in Risk Assessment* (NRC, 1994). The report also stated that agencies should have principles for choosing default options.

When pathogen-specific data are unavailable (i.e., when there are data gaps) or insufficient to estimate parameters or resolve paradigms, a default can be used in order to continue with the risk assessment. This is a science-policy choice, generally agreed upon during the planning and scoping discussions, when data gaps are identified (see section 2.4.4 for information on data gaps). During the risk assessment itself, a default is used only when essential data are lacking. Point estimates also can be considered defaults when the distribution of the parameter adds unnecessary complexity given the needs of the risk assessment. For example, drinking water consumption is often modeled probabilistically for MRAs with a median of 1liter per day. The consumption value of 2 liters per day per person is often used for chemicals and represents the 90th percentile of the 1994 to 1996 and 1998 *Continuing Survey of Food Intake by Individuals* community drinking water consumption data. As illustrated in this example, the choices you make need to be well within the range of plausible outcomes and often at specific percentiles (for variability) within that range of observation. The use of 1liter versus 2liters/day is not related to differences in microbial versus chemical risk assessment.

The default assumptions are not pathogen-specific *per se*, but are relevant to the data gap in the risk assessment. Defaults are based on published studies, empirical observations, extrapolation from related observations, and/or scientific theory. Appendix A of this volume provides a representative list of assumptions commonly made.

Beyond these two ways, this guideline does not address the types of policy or regulatory decisions that occur after the results of the risk assessment have been

considered. Science-policy decisions are differentiated from scientific judgment calls. Whereas risk assessors can make decisions based on scientific judgment, if a decision goes beyond what would reasonably be considered firmly supported by science, then policy comes into play. Once policy is involved, then risk managers need to become engaged in the decision-making. Failing to distinguish between policy decisions and scientific judgment in a risk assessment is a serious threat to the scientific credibility of the assessment. It is important to note that:

a) The utilization of science policy in the risk assessment process is not meant to "bury" or "hide" risk management decisions within the risk assessment. The use of a science-policy position or choice in the risk assessment process does not direct the risk assessment itself toward a specific risk management decision.

b) To be transparent, the risk assessment should state policy choices explicitly.

c) The policy positions themselves are developed outside the risk assessment.

Scientific data should support science-policy positions, and risk assessors and risk managers should ensure that the risk assessment proceeds in a way that provides the most accurate information for decision-making.

1.10 Why are Uncertainty and Variability in MRA Important?

According to the NRC, characterizing uncertainty and variability is key to the risk assessment process (NRC, 2009). The NRC provided recommendations on use of defaults, methods for, if possible, quantifying uncertainty, and how to consider variability in exposure and susceptibility. The NRC defines regarding uncertainty and variability in risk assessment (NRC, 2009):

> Uncertainty: Lack of or incomplete information. Quantitative uncertainty analysis attempts to analyze and describe the degree to which a calculated value may differ from the true value; it may use probability distributions. Uncertainty depends on the quality, quantity, and relevance of data, as well as the reliability and relevance of models and assumptions.
>
> Variability: Variability refers to true differences in attributes due to heterogeneity or diversity. Variability is usually not reducible by additional measurement or study, although it can be better characterized.

Almost every aspect of a risk assessment will have some level of uncertainty, usually due to data gaps and incomplete knowledge. Variability is a natural part of biological systems and will always exist. Often variability cannot be reduced, but it can be better understood with more information and knowledge. Both of these aspects are discussed in the risk assessment, and the degree of uncertainty and variability are characterized and quantified, if possible.

This guideline discusses ways to address uncertainty and variability (e.g., use of defaults, quantitative uncertainty analysis, sensitivity analysis, use of expert elicitation, or probability distributions). While there are techniques available to perform these analyses (e.g., two-stage Monte Carlo analyses), such analyses are not always necessary to address the particular risk management questions/issues at that moment. The "depth" or level of detail of these analyses can be discussed, particularly during the planning and scoping phase of risk assessment (e.g., see section 2.3).

1.11 Summary

This guideline is applicable to a wide array of scenarios but focuses on microorganisms that are capable of causing infection and disease in humans. Specifically, it is applicable for assessing risk associated with ingestion of foodborne pathogens (e.g., raw and processed foods), and water-based or waterborne pathogens (e.g., drinking water, recreational water, wastewater). Risk assessors could apply these guidelines to assess risks of human exposure to biological warfare agents or pathogens in soil, solid wastes, or air. This guideline also may apply to other common forms of exposure, including inhalation and dermal exposure pathways. At present, this guideline does not cover oligonucleotides, prions, preformed microbial toxins, and other submicrobial entities owing to a wide array of unknowns associated with those agents.

This guideline accounts for differences between the general population, different life stages, and sensitive populations. It is flexible to allow risk assessors to address risks to individuals, populations, and the general population using either available static or dynamic susceptibility models. This guideline provides approaches and tools appropriate for typical human health related concerns. The guideline does not include criteria to identify sensitive groups or life stages, because those groupings are specific to each risk assessment, based on the agencies' unique health protection concern and public health goal.

This guideline facilitates systematic and transparent consideration of relevant factors that impact the risk assessment and facilitates reproducible risk evaluation. This process allows agencies assessing a similar medium or pathogen to compare and contrast the details and assumptions of their assessment to another agencies' assessment. This is not to say that each risk assessment will be completely cross-comparable, because there are a number of specific data sources and agency requirements that require different inputs and applications. The differences in requirements are why this guideline is designed to be modular and able to provide flexibility for each agency's specific requirements. On an international scale, there also is a need to have common approaches to an MRA to effectively satisfy international trade agreements and public health protection for importation of food and beverage products and assess international risks from emerging pathogens around the world. This guideline is harmonized with both the NRC and Codex frameworks.

2. PLANNING AND SCOPING

Planning and scoping ensures that a risk assessment is relevant and well done. The NRC 2009 framework recommended "increased attention to the design of risk assessment in its formative stages [and] that planning and scoping and problem formulation, as articulated in EPA guidance documents (EPA, 1998a, 2003a), should be formalized and implemented in EPA risk assessments" (NRC, 2009). Rigorous preparation is needed at the start of the risk assessment process to facilitate communication during and following the risk assessment and to ensure that all issues are sufficiently vetted, all participants are clear on the objectives and goals, and managers are clear about the commitment of personnel and other resources (EPA, 1992; NRC, 1996).

To obtain detailed descriptions of the usefulness and implementation of the planning and scoping process, the FDA, USDA, and EPA provide general information on how to proceed (FDA, 2002; USDA, 2003a; EPA, 2000a, 2002a; NRC 2009). This chapter provides an overview of this first step in the risk assessment process. Several interesting case studies from ecological risk assessment are presented in EPA's *Lessons Learned on Planning and Scoping for Environmental Risk Assessments* (EPA, 2002a).

2.1 What is Planning and Scoping?

Planning and scoping is a process that defines the purpose and scope of a risk assessment and focuses the issues and approach(es) involved in performing the assessment. A clearly articulated purpose and scope provides a sound foundation for later judgments on the success of the risk assessment and for an effective risk characterization. In a sense, the planning and scoping process lays out a "road map" for how the risk assessment will be accomplished (Text Box 2.1).

Text Box 2.1. Planning and Scoping (based on EPA, 2003a, 2004a; NRC, 2009)

Planning and scoping involves:

- 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 (e.g., will the risk assessment include static or dynamic modeling)
- Agreeing on resources available and schedule
- Formulating the problem
- Developing the conceptual model
- Constructing the analysis plan
- Identifying initial risk management options that are available

The planning and scoping process helps all parties involved in the risk assessment understand how the risk assessment fits into the overall decision-making process. Planning and scoping promotes:

a) Identification of appropriate timelines and needed resources, thereby improving efficiency;

b) agreement among principle parties on realistic expectations regarding the goals, commitment, time-frame, and resources;

c) the prospect of less unanticipated controversy, because all interested parties contribute and disagreements can be dealt with swiftly and not left as a surprise at the end;

d) identification of and participation by those from many disciplines (e.g., microbiologists, toxicologists, economists, lawyers) to help in the process thereby ensuring that each risk assessment and characterization is useful for the intended audience(s);

e) an understanding of the degree of complexity needed in the risk assessment to adequately inform the decision at hand; and

f) informed decisions with stakeholder buy-in.

2.1.1 What is Problem Formulation?

At the beginning of the risk assessment, and frequently within planning and scoping, a problem formulation exercise frequently occurs. It is a discussion and analysis activity that focuses the technical/scientific aspects of the issue at hand. All relevant parties, including the risk manager, risk assessment team, risk communication specialist, and, when appropriate, relevant stakeholders and interested parties, participate. Problem formulation usually provides:

a) A definition of the valued entity and endpoint: what is the entity that should be protected and what are the undesirable effects that you are trying to avoid. For MRA this may involve a policy determination of what the "valued entity" is (e.g., general population, young children, pregnant women, immunologically compromised) and what is considered to be an appropriate level of protection (ALOP) against infection or disease.

b) A conceptual model that lays out the anticipated exposure scenarios of the microorganisms from the source to the receptor. With an MRA, this may be the movement of enteric pathogens from a source (e.g., treatment works, manure application to a field, critical point in the food processing system) to the population of concern. It highlights various hazardous events that may lead to increased risk and where risk management may be most effective.

   In some cases, a valid conceptual model may be unavailable, or there may be multiple plausible, but distinct conceptual models. The validity of a selected conceptual model may simply be uncertain. The risk assessor should be aware of these possibilities and may need to consider multiple (and uncertain) conceptual models as well as possibly model-free (e.g., source tracking) methods as part of the formulation.

c) An analysis plan provides a road map for addressing the problem. In short, it is analogous to an experimental design. In the analysis plan, risk hypotheses generated earlier are examined and discussed; the relationships between pathways and valued entities are further examined. The level of precision and data quality is considered in light of available information. The analysis plan may not be restricted to only pathway-based approaches because all important pathways may not be known with confidence.

It is important in problem formulation to identify as many possible (more importantly, probable) outcomes and their consequences. Also, unintended consequences should be identified where possible and addressed as part of the scope of the MRA (e.g., do possible interventions targeted at reducing one pathogen run the risk of increasing illnesses from another pathogen, or do the mitigation strategies themselves have risks [e.g., disinfection byproducts]). During the problem formulation activity, a "bad" formulation could ultimately restrict the value of the subsequent risk assessment.

2.2 What do I Consider When Deciding to Initiate an MRA?

Unless your agency is required to conduct a particular risk assessment (e.g., statutorily mandated), managers need to decide whether a risk assessment is appropriate, feasible, and will actually be performed. This decision is commonly made during planning and scoping. The agency may decide not to initiate a risk assessment after the planning and scoping step because a decision can be reached without conducting a risk assessment. Several criteria to consider for identifying a candidate risk assessment include:

a) Characteristics and importance of the hazard(s) of concern;

b) Magnitude (e.g., presence, prevalence, concentration of hazards) and severity (e.g., impact on public health) of the risk;

c) Urgency of the situation;

d) Populations of concern;

e) Other factors associated with specific hazards (e.g., water treatment processes, food processing, cooking, cross contamination);

f) Availability of resources (e.g., time, money, staff).

A diversity of ideas at the planning and scoping stage is important for exploring the range of possibilities (i.e., whether a risk assessment is truly needed to what type of risk assessment may be necessary). It is also important not to focus on one aspect too soon during planning and scoping as that may not be the "best" or "correct" way to proceed.

Whether a risk assessment may be required to comply with regulatory analysis requirements (OMB, 2003) is another consideration to take into account during planning and scoping. The Office of Information and Regulatory Affairs has provided a checklist to assist agencies in producing regulatory impact analyses (RIAs), as required for economically significant rules by OMB Circular A-4 (OMB, 2010; OMB, 2003). Appropriate assessments of risk may be necessary to address international trade agreements (e.g., World Trade Organization Sanitary and Phytosanitary Agreement). Ensuring that each assessment of risk is fit for its intended purpose and is based on scientific data most relevant to the national context ensures that the effort and scope of the assessment of risk are appropriate for the risk management questions being raised so that practical risk management options can be formulated.

Text Box 2.2 Examples of when a risk assessment may be appropriate.

- Review of the reliability or utility of a standard
- Cases where the current standard is inconsistent with other government policies, guidelines, or thresholds
- Cases where an agency has been petitioned for a regulatory action
- Establishment of standards for regulatory action
- Evaluation of the public health implications of different tolerable risk levels
- Cases where a data gap analysis is desired
- Cases where the hazard is a serious health issue, emerging pathogen, and/or public health concern
- The exposure system is complex.

Text Box 2.2 provides a list of examples of when a risk assessment may be appropriate. Text Box 2.3 provides FDA Center for Food Safety and Applied Nutrition's (CFSAN) process for selecting risk assessment topics.

Text Box 2.3 FDA CFSAN's four phases for selecting, conducting, and communicating food safety risk assessments (FDA, 2002).

Phase 1: Concept Generation – Collect ideas and maintain a list of potential risk management questions for which a risk assessment would assist with policy decisions. Develop justification for candidate risk assessments, including purpose of assessment, scope of problem, importance to the Center, and use of the result by the Center.

Phase 2: Problem Identification – The candidate risk assessment and supporting information (justification) are reviewed to determine whether the assessment meets the Center's needs. This phase results in one of three recommended actions, conduct data feasibility study, not required for regulatory decision, or more information needed to make decision.

Phase 3: Data Feasibility (Evaluation and Recommendation) – Information is collected and reviewed to determine availability of data needed to answer risk assessment question(s). This phase results in one of four recommended actions, conduct quantitative risk assessment, conduct qualitative risk assessment, more research needed, or modify question and conduct alternative assessment.

Phase 4: Disposition (Selection) – Using the results from the data feasibility determination as an aid, risk assessment(s) to be conducted are selected. Decision is based on technical merit, resource availability, the Center's priority needs, and other legitimate factors.

2.3 What "Depth" Can I go into in the Risk Assessment?

Once a decision is made to initiate a risk assessment, a major consideration for the risk assessment approach is how much detail or "depth" to incorporate to address the risk management question(s) or decision. Due to various management needs, the risk assessment approach is not necessarily one-size-fits-all. This guidance is intended to provide flexible methods for supporting different types of assessments (e.g., screening, safety) and outputs (e.g., qualitative or quantitative), as described in Schaffner (2008). Guidance from Codex (1999) and World Organization for Animal Health (OIE, 1999) described qualitative and quantitative outputs as equally valid (Wooldridge, 2008). Wooldridge (2008) provides detailed discussion of qualitative and quantitative assessments, and other risk analysts (Dennis et al., 2008) discuss both estimates of risk and safety, mortality for listeriosis, and allowable ('safe') levels of *Vibrio* in seafood, respectively.

Be aware that the terms referring to the different types of assessments are specifically defined in different contexts; care should be used when "naming" or referring to types of assessments. For example, this guideline is specific to the selection and conduct of risk assessment, not a safety assessment. One difference is that risk

assessment estimates the likelihood and/or frequency of adverse health outcomes resulting from an exposure and, in some cases, sources of risk. It also deals with quantitative reductions in risk based on various interventions. While much of this guideline is relevant to a safety assessment, a safety assessment may estimate the likelihood and/or frequency of exceeding a specified threshold of concern (e.g., predetermined regulatory limits or standards) or provide a determination of what is "safe" based on the conventions of the standard-setting procedure. For example, Codex standards traditionally specify maximum limits for additives and residues in foods based on the concept of "no appreciable risk" (FAO/WHO, 1997). For contaminants in foods, the Codex standards are based on the concept of "as low as reasonably achievable" (FAO/WHO, 1997). Risk-related terms in some statutes have formal definitions. The risk assessor should be aware of statutory definitions that apply to a risk assessment.

There are many cases when a screening risk assessment is preferable over a fully developed risk assessment (see section 1.8 for screening definition). It is important to be transparent about the amount of uncertainty in the screening estimate and discuss whether the uncertainty causes underestimates or overestimates of risk based on the assumptions applied. This work may be followed by a more detailed assessment that will need to be conducted or risk managers will need to take action(s). The risk assessor may return to the assessment to refine and recalculate estimates based on additional data (e.g., quick sampling assays, use of surrogate data, expert elicitation). Alternatively, there may be a risk management decision to conduct a full risk assessment.

A comprehensive ("major") risk assessment requires a substantial commitment of resources. Thus, this depth of risk assessment is not necessary when risk managers do not need this level of sophistication to make a decision. Circumstances that may not warrant a quantitative risk assessment would include, for example, a risk that is well described by definitive data, a problem that is relatively simple, or an issue that is not of regulatory concern. However, a comprehensive risk assessment is a powerful tool to help risk managers evaluate and interpret information when the data describing a hazard are incomplete, the exposure system is complex, or the issue is of high regulatory or stakeholder concern.

Risk assessments can be either qualitative or quantitative (or parts of each, e.g., as in "semi-quantitative") in their description of the likelihood of adverse health effects, depending on the extent of the data and knowledge available, the existence of models or other tools for quantitative predictions, the complexity of the problem, the scope and nature of the question(s) posed by the risk managers, and the time available to conduct the assessment. In quantitative assessments, the risk is expressed as a mathematical statement of the probability of illness or death after exposure to a specific hazard, and it represents the cumulative probabilities of certain events happening and the uncertainty associated with those events. Conversely, qualitative risk assessments use verbal descriptors of risk, severity, and uncertainty, and often involve the aggregation of assumptions.

2.4 What Elements are Discussed During Planning and Scoping?

Various elements are discussed during planning and scoping. Not all of these elements are necessary for every risk assessment, and certain elements will be more appropriate for a particular problem. Examples of products of planning and scoping are listed in Text Box 2.4 and underlined in the text where the elements and activities of planning and scoping are described. Not all of the elements and activities that occur during planning and scoping have associated written products. This section begins with an overview of the elements and activities of planning and scoping, and then provides more detail for selected elements as third level header questions and answers.

Text Box 2.4 Products of Planning and Scoping

Statement of Concern
Statement of Purpose and Objectives
Background Section
Scope
Scenarios
Literature Review
Data Inventory
Tools and Methods Inventory
Risk Management Questions or Charge
Risk Profile
Conceptual Model
Value-of-information analysis
Communication Plan
Analysis Plan
Work Plan
Data Quality Objectives

Principal outputs from planning and scoping can include various products that are appropriate to the management objectives and the plan for analysis of the risk. The level of deliberation and content of the outputs vary depending on the complexity required for the assessment. Further, some outputs can be contained in other products (e.g., the risk profile document can contain many of the listed products). You need to discuss what outputs will be generated during the planning and scoping deliberations. In general, many of these products are good candidates for peer review. Peer review or consultation early in the risk assessment process can provide timely insights, corrections to assumptions, and directions on proper ways to proceed during the risk assessment.

During planning and scoping, participants can engage in a dialogue to answer the following questions and commit to the outcome of those discussions to facilitate mutual understanding (elements underlined):

a) **What is the motivation for the risk assessment?** A <u>Statement of Concern</u> relays a common understanding of what broad issue the risk assessment will address. Describe in simple terms what hazard is being addressed and how it is thought to relate to human health for an exposure scenario. Include any other driving factors for the risk assessment, such as a food safety issues, regulatory requirements, public concern, or new scientific findings.

b) **What are the management goals, issues, questions, and policies that need to be addressed?** A <u>Statement of Purpose and Objectives</u> is a concise paragraph that addresses the management goals. The management questions are designed to provide the information needed for decision-making. It is in the best interest of all parties involved (e.g., assessors, managers and decision makers, stakeholders)

to state, at the outset, the question(s) that needs to be answered in the assessment explicitly.

c) **What is the context of the risk assessment?** Risk assessments are done in historical and social contexts. A Background Section summarizes any previous risk assessments that addressed the same or similar hazards. A risk assessor can use a previous risk assessment to summarize the relationship between the current and previous risk assessment. The context of a risk assessment may include different mandates, regulatory requirements, policy developments, or information derived from technical advancements, risk assessment method and tool advancements, and new or enhanced data sets.

d) **What is the scope and coverage of the risk assessment?** The Scope outlines the scenarios the risk assessment will cover. Answering the scoping questions below can ensure the information regarding scope is necessary to conduct the MRA.

1) Which infectious disease hazard is being addressed (pathogen strain[s], indicator[s], or taxon [genus, species, strain/biovar])? Define the hazard.

2) Which human populations will be the focus of the risk assessment (e.g., general population, life stages, or geographically defined populations)? Describe which populations are explicitly included in the risk assessment model, which populations 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, and how is the health outcome measured? Clearly defining the health endpoint is important for transparency and 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? Determine the time-span of exposure relevant to the decision.

5) For risk assessments designed to derive nominally or presumptive “safe” levels of microorganisms (i.e., levels below a threshold of regulatory concern), what level of protection will be provided, and what is the technical or policy justification for that level? Transparency in public health objectives is important.

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

e) **What type of risk assessment is needed to address the risk management question(s)?** Section 1.8 describes different types of risk assessments, including screening, risk ranking, product pathway, risk-risk, geographic, systems-level (sustainability risk assessment), and threat and vulnerability assessments.

f) **What is the state of the current knowledge?** The planning and scoping can include an overview of current knowledge and be used to outline the topics that will be reviewed in more depth in the rest of the risk assessment. A Literature Review provides understanding of the current state of the science.

g) **What and where are the available data?** In addition to identifying the available data, a Data Inventory addresses data relevance, data use, data accessibility, and data quality. An inventory may also provide notice to the public of the data currently available to the agency in a call for data. In addition to literature searches, government databases provide data that are useful for risk assessments. For example, WHO maintains the Global Health Observatory Data Repository, the Centers for Disease Control and Prevention (CDC) conducts the National Health and Nutrition Examination Survey (NHANES), as well as the FDA and the University of Maryland's Joint Institute for Food Safety and Applied Nutrition sponsors foodrisk.org.[2] See section 2.4.6 for further discussion of data quality.

h) **How do I know what questions the risk assessment needs to answer?** The Risk Management Questions or Charge are usually written down and discussed iteratively between the risk assessment team and risk managers to ensure common understanding of the questions. See section 2.4.1 for discussion on risk management questions and charge.

i) **What are the information/data needs of other members of the "team?"** The risk assessment may be part of a larger project, such as an economic analysis. There may be economic, social, or legal analyses that need to be coordinated with the risk assessment.

j) **How will you model the risk?** A Tools and Methods Inventory typically includes statistical methods for estimating model inputs and tools for addressing uncertainty and variability and should make an initial determination of which methods and tools are likely to be most useful. It is advised that models used in the risk assessment be peer reviewed preferably before starting the risk assessment. Software needs should also be considered; some risk assessment software tools include Oracle's Crystal Ball, Palisade Corporation's @Risk, Berkeley Madonna, and Lumina Decision System's Analytica. EPA's *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* has citations for many tools (EPA, 2007c).

k) **What are possible risk assessment or risk management options?** Particularly for risk assessments that are needed to evaluate intervention strategies or needed to support regulatory determinations, different options should be presented as different scenarios for the risk assessment. Those Scenarios should be clearly stated during problem formulation and may evolve during risk assessment iterations.

[2] http://apps.who.int/ghodata/ ; http://www.cdc.gov/nchs/nhanes.htm;http://foodrisk.org/

l) **What are the logistical considerations for conducting the risk assessment?** The Analysis Plan (see section 2.4.5) or a Work Plan contains logistical considerations. These include:

1) resources available to do the assessment, including funding and staff time;

2) participants in the process and their roles;

3) plans for coordinating across offices, with other agencies, and with stakeholders; and

4) scheduling (e.g., milestones, deliverable due dates, quality audits, meetings), including provisions for timely and adequate internal, independent external peer review, and if required, interagency review.

m) **How will planning activities and results be communicated to senior managers and to the public?** Chapter 8 discusses risk communication within the context of risk assessment.

n) **What are the legal considerations and constraints that may shape the ultimate decision and supporting risk assessment?** The technical office conducting the risk assessment can engage the agency's legal department, according to normal practices within that agency. The Background Section discusses legal and statutory context.

o) **How and at what iterations will the risk assessment be peer reviewed?** OMB has published *Information Quality Bulletin for Peer Review*, which provides general peer review guidance and sets minimal expectations for the review of scientific information (OMB, 2004). Most agencies have agency specific peer review guidance that complies with the OMB guidance. Follow your agency's peer review guidance. For example, EPA's *Peer Review Handbook* (EPA, 2000c, 2006c) 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:

1) training and experience in the subject area resulting in superior knowledge in the field;

2) access to relevant information;

3) an ability to process and effectively use the information; and

4) 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.

The following sub-sections flesh out some of the major elements iterated above.

2.4.1 What are Risk Management Questions and What is the Charge?

Section 2.4 (above) presents a brief description of management questions. Generating specific risk management questions for a risk assessment helps formulate a clear, focused charge that identifies the technical and scientific issues on which you need to address and suggestions for conducting the risk assessment. Formulating these questions usually requires significant interaction between risk assessors and risk managers, as well as dialogue with appropriate other parties (e.g., those with relevant information about the potential hazard). The questions generated will focus the risk assessment to provide the appropriate analyses to inform the risk management decision at a level of detail appropriate for the issue. The resulting risk assessment can be designed to address and answer as best as possible the risk management questions posed.

The charge focuses the assessment by presenting specific questions and concerns, including the comprehensiveness of the data, information, and literature, the soundness of the methods proposed, the scientific support for the assumptions employed, and the sensitivity of the results to possible alternative assumptions. In general, time is well-spent preparing a good set of questions or a charge, which are crucial for an effective risk assessment and ultimate decision. In this context, the charge is the set of questions and does not imply a formal charge.

2.4.2 What is a Risk Profile?

Codex defines a risk profile for food safety as: "a description of a food safety problem and its context that presents in a concise form, the current state of knowledge related to a food safety issue, describes potential microbiological risk management options that have been identified to date, when any, and the food safety policy context that will influence further possible actions… Consideration of the information given in the risk profile may result in a range of initial decisions, such as commissioning a microbiological risk assessment, gathering more information or developing risk knowledge at the level of the risk manager, implementing an immediate and/or temporary decision" (Codex, 2007a).[3] A typical risk profile includes: a description of the situation, product, or commodity involved; information on pathways by which consumers are exposed to the microorganism; possible risks associated with that exposure; consumer perceptions of the risks; and the distribution of possible risks among different segments of the population. For a list of information that Codex recommends including in a risk profile see Codex (2007a).

A risk profile assists in identifying the risk management questions that need to be addressed. The risk profile should be clearly and thoroughly documented, so that risk managers can use it to decide on further action in relation to a specific health issue. If

[3] "Risk profile" is sometimes used to refer to summary information at the end of each chapter in frameworks based on the ecological risk assessment framework.

links are made between risk profiles for other risk assessments, risk profiles can provide the basis for qualitative ranking of problems for subsequent risk management.

Notably, risk profiles can be used as decision tools that do not lead to risk assessment. For examples, see the New Zealand Food Safety Authority.[4]

2.4.3 What is a Conceptual Model?

A conceptual model is a written or visual representation of predicted relationships between the hazard and exposed populations.[5] It is based on problem formulation and working hypotheses; it is supported by preliminary data and information and used to organize the conduct of an MRA.

The conceptual model depicts the movement of the hazardous agent from the source to the host. Other tiers of conceptual models may identify variables and data needed to conduct the MRA. A conceptual model (e.g., a source-pathway-receptor model; "farm-to-fork" model) can be developed early in the planning and scoping process, to the level necessary to address the risk assessment's purpose. In some cases, a pathogen may be available in multiple media and cause different diseases depending on the route of exposure. During planning and scoping, the risk assessor can assess the medium, pathway of exposure, and route of exposure. An example of a conceptual model is provided in Figure 2.1. Other examples of conceptual models can be found in Suter (1999).

Developing a sound and useful conceptual model may require several iterations. The conceptual model describes or visualizes the relationships among the assessment and measurement endpoints, the data required, and the methodologies that will be used to analyze the data. An overall high-level conceptual model as well as more detailed conceptual models that cover just dose-response or exposure assessment components may be useful (EPA, 1998a). Overall, the purpose of a conceptual model is to enhance the documentation of the risk assessment so that readers will have a clearer picture of the risk assessment.

[4] http://www.foodsafety.govt.nz/science/risk-profiles/

[5] The conceptual model in planning and scoping differs from conceptual models that are used to map how parameters are related in modeling software. The term conceptual model is used in both contexts in this Guideline.

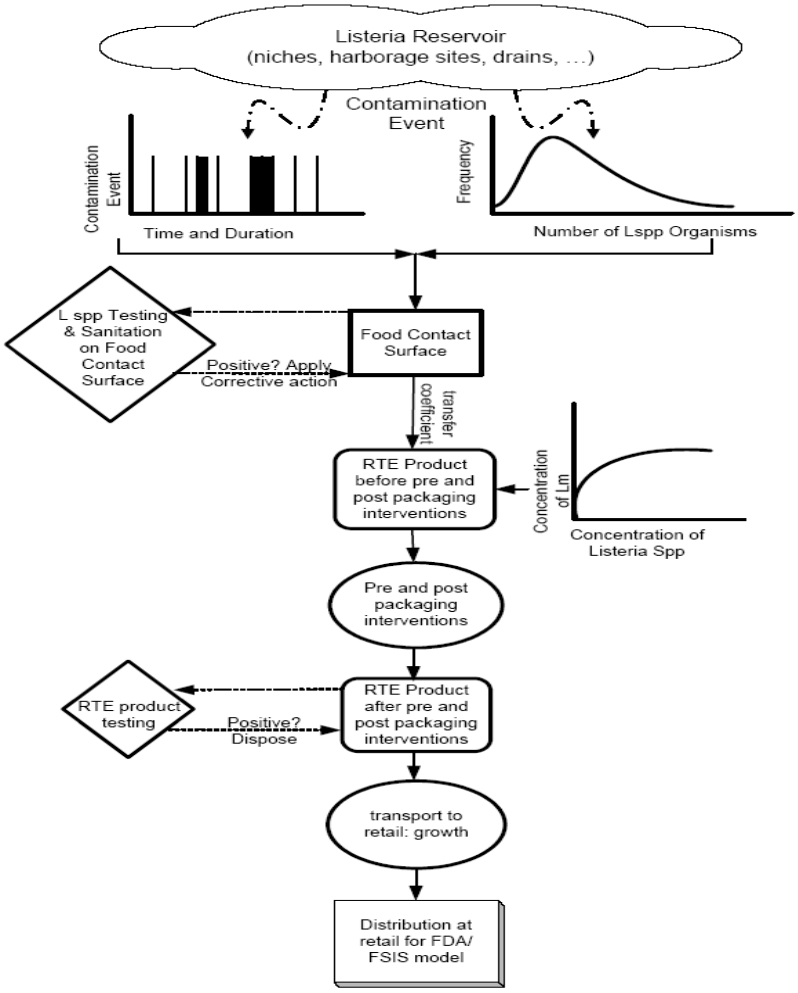

Figure 2.1 Conceptual Model for the "in-plant" component of the *Listeria* risk assessment (from USDA 2003b).

2.4.4 How are Data Gaps Identified and Addressed in the Context of Planning and Scoping?

Incomplete information and data gaps are a significant challenge throughout the risk assessment discipline. Much of the "art" in risk assessment involves the judgments regarding incomplete data and data gaps. In addition to missing data, risk assessors must assess whether available data are representative of the actual conditions.

The extent to which a data gap exists is ultimately a matter of scientific judgment within the context of what is an acceptable level of confidence. Different assessors and managers may have different comfort levels for making decisions based on the same data. In some cases, there may be differing options about how representative the data are and whether the data adequately fit in the risk assessment scenario. The risk assessor must

consider the quality of existing data when determining if a data gap exists. To reduce the heterogeneity of comfort levels, many different systematic schemes for evaluating data quality, completeness, and applicability have been developed. Statistical approaches are standard for evaluating data quantity, but still require a judgment about the appropriate confidence level for decision-making. If the risk assessors and risk managers on your team cannot agree on what constitutes a significant data gap, the team may need to take a step back and first agree on criteria for evaluating data. With some data gaps, risk managers look to risk assessors to tell them whether the data are sufficient, while risk assessors may claim that a policy decision needs to be made by the risk managers regarding setting the threshold for sufficiency. These types of situations can stall a risk assessment, but ultimately the group has to reach agreement on the judgment call or policy decision. If the existence of a data gap is not obvious and agreed upon easily, the risk assessors and managers should document the evidence used to support the identification of the data gap.

Stakeholders may have strong opinions about data gaps. Some may demand a decision in the absence of data, while others may interpret the goal of science-based decision-making to mean that more data must be available to make a decision. Robust planning and scoping should be able to predict which data gaps have the potential to cause the most debate.

Every parameter in the risk assessment will have some level of incomplete information. Ranking the importance of the data gaps can help focus resources on the most critical data gaps that, if filled, could influence the risk assessment results the most. If there is more than one data gap, it may be critical to examine the set of gaps and their possible interdependence when deciding how important the gaps are to the risk assessment. The risk assessment results can be evaluated by conducting sensitivity analysis (sections 5.3.3 and 6.6) or value-of-information (VOI) analysis (section 2.4.7) to determine whether the data gap is significant. Determining data gaps and the relative importance of different data gaps will progress iteratively as the risk assessment is conducted.

Once a data gap (or set of gaps) is identified and determined to be important, it can be a matter of scientific judgment or a policy decision that determines how the data gap(s) will be addressed in the risk assessment. Use existing data in the near term to fill information needs; in the midterm, conduct tests with currently available test methods to provide data on the topic of interest. Over the long term, develop better, more realistic understandings of exposure and effects and construct more realistic test methods to evaluate pathogens of concern. In cases where an aspect of risk is likely to be important but insufficient data are available, highlight the deficiency or use judgment or assumed values to approximate the missing data (see section 6.4 for further discussion). The risk assessor should clearly describe the judgments and approximations, and the implications explained in the risk characterization.

OSTP states in their scientific integrity memo that, “The accurate presentation of scientific and technological information is critical to informed decision making by the

public and policymakers. Agencies should communicate scientific and technological findings by including a clear explication of underlying assumptions; accurate contextualization of uncertainties; and a description of the probabilities associated with both optimistic and pessimistic projections, including best-case and worst-case scenarios where appropriate" (OSTP, 2010).

Depending upon the circumstances, the utility of a risk assessment may be compromised if important policy decisions are put on hold while waiting for more research results. Risk assessors and risk managers need to balance the need to obtain more data/information against the need to make a timely decision. The data gaps identified in planning and scoping may be very useful to establish a research program and/or agenda to address current data gaps.

When data or information are lacking, expert opinion or judgment is an alternative source of information, particularly used in exposure assessments. If no other empiric evidence is available, expert judgment may offer insights to inform a model for example. On the other hand, when data are completely absent and the availability of expert opinion or judgment is questionable, it is possible to avoid the need for such data by model simplification (Cox, 2006; Vose, 2008). Such an approach is particularly worthwhile when empiric evidence is available to inform the probability distributions of process outputs subsequent to the process that is missing data. Such 'downstream' data (i.e., between the missing element and the ultimate exposure distribution) actually reflect the likelihood of microbe levels given the processes (e.g., modeled or missing) that occurred prior to the process. Therefore, processes for which data are missing may effectively be skipped over if data are available downstream.

Methods for eliciting expert judgments have been suggested (Kaplan, 2000; ECSCC, 2003). Ouchi (2004) and Morgan and Henrion (1990) provide summaries of methods and citations for primary literature in the field of expert elicitation. Techniques for resolving conflicting opinions among experts focus on having experts cite the experiences that inform them. In general, a diverse group of experts is preferred when eliciting input to the exposure assessment.

Hoffmann et al. (2007), for example, used expert elicitation to attribute illnesses associated with one of eleven major foodborne pathogens to the consumption of one of eleven categories of food. They used responses from a large panel to create and analyze four uncertainty measures: (1) agreement among experts; (2) expert agreement with prior estimate; (3) mean individual expert uncertainty; and (4) variability in experts' individual uncertainty. Hoffmann and colleague's framework shows how these measures when viewed together can provide greater insight into the state of knowledge available to support decisions than could individual measures. They used statistical analysis to assess the quality of both expert judgment data and external data. Hoffmann and colleagues suggest that analysis of multiple uncertainty measures is likely to be particularly useful to decision makers when external validity checks that rely on conventional scientific methods or further data collection are infeasible or costly.

2.4.5 What is an Analysis Plan?

The analysis plan is the implementation strategy for performing the risk assessment and addressing the decision needs. The analysis plan lays out the approach to be taken by the risk assessment team. It shows how risk assessors use and integrate data sources and information into the assessment and how measurement endpoints (e.g., fecal shedding) and uncertainties are related to the assessment endpoints (e.g., morbidity and mortality). As a product of planning and scoping, the analysis plan can act as a bridge to the risk assessment. It documents the agreements made during the planning and scoping process and provides details on how the risk assessment will proceed. This step provides transparency to the whole process. In addition, the analysis plan provides measures to evaluate the final risk assessment and its risk characterization. As the risk assessment proceeds, the analysis plan may need revision to ensure that the risk assessment still meets the decision needs (EPA, 1998a). In the absence of a separate work plan for logistics, the analysis plan can include the staffing, scheduling, and resource details. As noted above, peer review or consultation of the analysis plan can provide valuable input at a critical stage of the assessment process.

2.4.6 How do I Consider Information Quality Including Data Quality?

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") directed 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." Federal agencies responded to the OMB guidelines (OMB, 2002) by developing agency specific guidelines. For example, EPA published *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of Information Disseminated by the Environmental Protection Agency* (hereafter known as EPA Information Quality [IQ] Guidelines; EPA, 2002b). EPA's IQ Guidelines[6] include the following adaptation of the quality principles found in the Safe Drinking Water Act (SDWA) Amendments of 1996 (EPA, 2002b):

a) The information is accurate, reliable and unbiased. This involves the use of:
    1) the best available science and supporting studies conducted in accordance with sound and objective scientific practices, including, when available, peer reviewed science and supporting studies; and
    2) data collected by accepted methods or best available methods (if the reliability of the method and the nature of the decision justify the use of the data).
b) The presentation of information on human health, safety, or environmental risks, consistent with the purpose of the information, is comprehensive, informative,

[6] These principles should be adopted or adapted in all Federal agency IQ Guidelines for assessments related to evaluations and public heath (OMB, 2002).

and understandable. In any document made available to the public, the IQ Guidelines specify that the following information needs to be included:

1) each population addressed by any estimate of applicable human health risk or each risk assessment endpoint, including populations if applicable, addressed by any estimate of applicable ecological risk;
2) the expected risk or central estimate of human health risk for the specific populations affected or the ecological assessment endpoints, including populations if applicable;
3) each appropriate upper-bound or lower-bound estimate of risk;
4) each significant uncertainty identified in the process of the assessment of risk and studies that would assist in resolving the uncertainty; and
5) peer-reviewed studies known to the administrator that support, are directly relevant to, or fail to support any estimate of risk and the methodology used to reconcile inconsistencies in the scientific data.

Discussion of data quality is an important part of planning and scoping. You can evaluate data quality within the context of your agency's 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 may differ depending on the planned use of the risk assessment. If data are excluded, it is always important to note the exclusion and reason for the exclusion. Methods for evaluating data quality are important tools for producing a risk assessment that has both scientific value and credibility with stakeholders.

It is recommended that Data Quality Objectives be established that are consistent with agency policy. Data Quality Objectives facilitate transparent documentation of the justification of why data were included or excluded (IRAC, 2000). The following list describes several important characteristics that may be helpful to evaluate the usefulness of data sets for risk assessment, such as submitter information, data source, methods, and confidentiality (adapted from IRAC, 2000):

a) General information: Complete name and correspondence address of principal investigator, purpose of study, and availability of raw data;
b) Source of data: Funding source/affiliation of principal investigator or data collectors, who collected/produced the data, and for numerical data provide numerator and denominator study design (e.g., type of study, sample size, sampling frame/sample selection, and how sample relates to the population [is the sample from a particular country, region or producer?]);
c) Data collection: Method of data collection/compilation, age of data, country/region of origin, time frame for collection (seasonality), and conditions of collection (field versus laboratory data);
d) Microbial methods: Testing methods (i.e., tests were run), sensitivity and specificity of test(s), techniques used, precision of measurement, definition of

units being used, species of animals used, if any, and specific organism tested or studied;

e) Evaluation of information: Consistency with regard to findings of other researchers, publications that cite the data, peer review of the data, investigator's evaluation of data, and investigator's recommended limitations of data; and

f) Protections for sharing raw data: Confidentiality for human subjects (blinded data).

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, 1998a):

a) 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, and route).

b) Are the variables and conditions in the study comparable with those important for the risk assessment?

c) Is the study design adequate to meet its objectives?

d) Was the study conducted properly?

e) Were there associations between observable data and the outcomes (health or otherwise) of interest?

f) Does the data control for factors that could increase or attenuate risk (risk factors)?

g) How are variability and uncertainty treated in the study report?

h) Are the data sufficiently robust to support a causal effect between exposure and infection or illness?

i) Does the study meet agency requirements regarding ethics, such as having passed internal review boards or complying with agency regulations regarding research? For example, EPA's Protections for Subjects in Human Research Rule[7] requires that all pregnant women, all nursing women, and all children are excluded from all studies involving intentional exposure that are intended for submission under pesticide laws. Additional information on the conduct and use of observational studies in EPA's risk assessments are addressed in *Scientific and Ethical Approaches for Observational Exposure Studies* (EPA, 2008).

[7] http://www.epa.gov/oppfead1/guidance/human-test.htm

EPA covers assessment factors, including soundness, applicability and utility, clarity and completeness, uncertainty and variability, and evaluation and review in their Science Policy Council *Assessment Factors* (EPA, 2003b).

Because data are never complete and are rarely collected specifically for risk assessment, risk assessors consider many types of data for a risk assessment. Quality data include complete datasets, relevant data, and peer-reviewed data that are considered high quality by experts in the field and agree with other data sets in terms of comparison of methods and development of tests. Complete datasets would include information on all the characteristics listed above. Relevant data may depend on the risk question under consideration. Some characteristics of relevant data include age of data, region or country of origin, purpose of study, and species involved.

2.4.7 What is Value-of-Information Analysis?

Planning and scoping discussions may address whether to wait for additional research or if available information is adequate. Value-of-information (VOI) analysis provides a set of methods for optimizing efforts and resources to gather, to process, and to apply information to help decision-makers achieve their objectives (i.e., provide insight when trying to resolve whether current uncertainties would increase the expected value of optimal decisions by more than the cost of that information). The NRC provides a schematic of the application of VOI analysis to assess the impacts of additional studies in a specific decision context (NRC, 2009). Information opportunities that address uncertainties in the baseline model are considered with respect to the changes they would have on the decision-maker's preferred decision option and the associated change in net benefits. For example, the aim of a VOI analysis for the decision maker will be in its ability to determine if more information about the risk of microbial pathogens is economically beneficial before making a decision (Disney and Peters, 2003). VOI analysis therefore provides a way to quantify the value of actions taken to reduce the risk associated with a decision (Hirshliefer and Riley, 1992).

2.4.8 What is a Communications Plan?

Risk communication (Chapter 8) can be initiated as soon as the risk assessment process begins and can be incorporated throughout the process; risk communication should not be an afterthought. During the planning and scoping discussions, the risk communication specialists need to work with risk assessors, risk managers, and appropriate stakeholders to develop a communication plan or strategy for communicating with the public (essentially all stakeholders). The plan should include:

a) clearly identifiable stakeholders and collaborators (e.g., in multiple partnering projects);

b) specific communication goals (e.g., what will be your proposed message[s]);

c) audiences, which can range from technically sophisticated risk assessors and knowledgeable risk managers to an educated lay audience with limited knowledge of risk assessment;

d) communication tailored toward your projected audiences;

e) proposed outreach avenues (e.g., fact sheets, press releases, newsletters, notices, open meetings, briefings, emails, websites).

Communication specialists, including your agency's public affairs office, distribute the message to the intended audience. It is advisable that these specialists be involved with the risk assessment effort from planning and scoping onward. Identify the principal spokesperson (possibly a risk assessor) and decide on information channel(s) to use. Your agency may have guidance for risk communication plans or stakeholder involvement. For further information regarding risk communication from an assessor's point of view, refer to Chapter 8.

2.5 Who Can be Involved with Planning and Scoping?

All interested parties could be involved with planning and scoping; however, bringing everyone together at one time and discussing all aspects of the effort at once may be difficult. This process may require several sessions. Major participants include relevant risk managers, risk assessors, and other members of the "team" working on the decision process. In addition to the appropriate microbiologists, the process should include infectious disease experts and/or individuals trained in infection control, and other members such as economists, lawyers, engineers, policy makers, and communicators.

A critical part of the planning and scoping dialogue is that among the risk managers and technical experts: risk assessors, risk communicators, economists, and other technical experts who develop the broad dimensions and elements of the risk assessment, the risk management questions and goals for the assessment, a tentative budget and schedule, and an approach for conducting the risk assessment. Risk assessors should be involved in every stage of planning and scoping, because risk managers will have technical and data related questions that may require input from the risk assessor. The risk assessor will want to weigh in on whether the tools and data available can answer the questions being posed. Planning and scoping would not be successful without the technical input of the relevant assessors and the management perspectives from risk managers. In addition, the risk manager should consider stating explicitly any reasons to limit the technical scope of the assessment. The risk assessor should consider including details on resource limitations, data availability and quality, and methods availability. In other words, you can be very clear and transparent about what you plan to include in the risk assessment and what you plan to exclude from the risk assessment and why.

The importance of stakeholder involvement during the planning and scoping process depends upon the nature of the problem, their interest, and ability to contribute.

Risk assessors should identify stakeholders early in the planning and scoping of the risk assessment. How stakeholders can be involved most effectively needs to be decided on a case-by-case basis. Public involvement, early and often, leads to a much clearer risk assessment product. This involvement also allows for flexibility and buy-in for future decision making if the need arises to deviate from the original plan.

Affected parties can share their perspective about the risk and risk management options. Their input is particularly helpful in determining what should be included in the assessment, personal risk or exposure, and additional data or exposure scenarios required.

Building the necessary relationships with stakeholders to maintain dialogue takes considerable effort, but this should not deter risk assessors and risk managers from engaging in this important activity. Refer to agency stakeholder involvement guidance to integrate these individuals into the planning and scoping process. Risk communication specialists should develop stakeholder involvement plans. The National Academies of Science report, *Public Participation in Environmental Assessment and Decision Making*, is a resource for considerations when engaging stakeholders (NRC, 2008).

2.6 Summary

In summary, the planning and scoping can set the basis for the success of the risk assessment process and the effectiveness of the management decisions. The planning and scoping discussion may include a preliminary characterization of exposure and effects, as well as examination of scientific data and data needs, policy and regulatory issues, and scenario-specific factors to define the feasibility, scope, and objectives for the risk assessment. It also establishes the level of detail and the information that will be needed to complete the assessment. Just as important, planning and scoping helps set the boundaries of the problem(s) addressed and the scope of the MRA.

For perspective, risk managers (decision makers) naturally desire more information, less uncertainty, and more in depth interpretation, when the impact of their decisions increases. They also want to know the financial and social implications of possible decisions. The risk assessment may not be the appropriate support analysis to address these issues, but if done well, risk assessment can be a critical input. These aspects may be discussed during planning and scoping.

3. HAZARD IDENTIFICATION AND HAZARD CHARACTERIZATION

Hazard identification and hazard characterization (HI/HC) are key components of risk assessment. In HI, the suspect microbiological agent and its associated adverse effects are identified and defined in the context of epidemiological, surveillance, clinical, microbial (agent specific), and environmental (including meteorological and geographical) information. The HC focuses on a particular microorganism(s) and potential or known mechanisms of host-pathogen interaction, virulence, pathogenicity and dose response. As discussed in section 1.4, the epidemiological triangle is a useful framework for conceptualizing HC. Meteorological and geographic conditions impact the persistence and transmissibility of microbial agents in the environment and influence the level of potential exposure through food and water.

This guideline document generally follows the Codex definition for the risk assessment process; in order to provide the necessary focus on the qualitative and quantitative modeling aspects, all the qualitative aspects of hazard characterization are combined with hazard identification in a single chapter—hazard identification and hazard characterization. The quantitative and modeling aspects of hazard characterization (dose response) are handled in a separate chapter that deals exclusively with dose response.

This chapter presents information that is basic to HI/HC. A list of questions that may be posed during HI/HC is presented in Appendix B.

3.1 What are Hazard Identification and Hazard Characterization?

HI and HC provide a *qualitative* examination of the hazard identified. The dose-response assessment (see Chapter 4) provides the *quantitative* relationship between hazard and effect. HI provides the framework for gathering relevant information to construct a realistic scenario focusing on the likely microbial hazards present appropriate for the MRA. HI reviews information related to the epidemiological, surveillance, clinical, and microbial aspects of the hazard as a critical part of the assessment. Hazard characterization helps you describe the mechanisms involved in causing harm and the microorganism's ability or potential to cause harmful effects.

HI, HC, and exposure assessment data vary greatly in MRAs. Coverage may vary depending on the type of the assessment (e.g., qualitative, quantitative, retrospective, prospective) and agency policy or convention. The extent of coverage and potential overlaps in the problem formulation step should be considered during planning and scoping. In this guideline, HC and some elements of exposure assessment are included in the HI/HC chapter.

3.2 How do I Define the Hazard?

The term hazard broadly refers to the subject of an assessment. It can be interpreted in a number of ways. Primarily, it may be defined as the stressor or agent capable of causing an adverse effect on the exposed individual(s). The subject of the risk assessment (hazard of interest) is a policy decision driven by the existing statutes, regulations, or consistency with agency processes. You should be aware that although the term hazard may broadly refer to an agent that is causal or associated with adverse outcome, the mechanisms and metabolic products leading to the outcome are important considerations. The adverse effects in humans that result from exposure to microbial agents or metabolites under favorable host (healthy or susceptible due to certain life-stages/pre-existing conditions) and environmental conditions may occur soon after exposure or arise at a substantially later time (sequelae). This guideline focuses on pathogenic infectious disease hazards.

Terms such as "agent" and "stressor" may sometimes be used synonymously with hazard.[8] The microbial Thesaurus developed by EPA (EPA, 2007b) differentiates the terms as follows: The term "'stressor' is used in ecological risk assessment and includes but is not limited to the connotation that the adverse response can be the result of a lack of something – such as a habitat – which would be called a 'stressor'. The term 'agent' does not have this connotation. 'Agent' is used to denote a causative entity that actually physically exists as part of the environment and can be used in either ecological or human health risk assessment. 'Hazard' is used primarily in human health risk assessment, although 'hazards' are not limited to 'agents.' For example, the number of days spent in a hospital may be a hazard that correlates with risk of nosocomial infection.

In the context of MRA for frank pathogens, the term hazard represents the pathogen's potential to cause adverse effects in normally healthy humans. In the case of opportunistic microorganisms, the term hazard refers to the potential to cause adverse human outcomes under certain environmental and host conditions, most often when the host is compromised. Thus, hazard may be used to denote the subject of the assessment that needs further identification and characterization.

If the hazard is a pathogenic microorganism, then identification of the microorganism is an important aspect of the hazard description. Microorganisms can be categorized (defined) based on the methods used to detect them. Nucleic acid-based assays may result in a different categorization than culture-based assays. As mentioned previously, quasi-species are particularly hard to define. It is important to note that in some situations even small DNA sequence changes may elicit significantly different adverse outcomes in humans (Battista and Earl, 2004). In the case of "zero tolerance" organisms (i.e., absence of pathogen), this distinction does not matter. Examples of the nuances of microbial nomenclature refer to section 3.8.

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[8] Epidemiologists may use the term "agent" slightly differently. Agent - a factor (e.g., a microorganism or chemical substance) or form of energy whose presence, excessive presence, or in the case of deficiency diseases, relative absence is essential for the occurrence of a disease or other adverse health outcome. http://www.cdc.gov/excite/library/glossary.htm

In general, when performing assessments on individual strains or isolates, identification rather than taxonomy or nomenclature becomes the issue. In this case, identification refers to the placement of an isolate within an existing taxon or determining that it does not match any existing taxon (i.e., proper labeling of the isolate), as opposed to the creation of a new group or groups to which the isolate is related, that share attributes with the isolate (i.e., classification of the isolate). Identification ensures the proper taxon or subtaxon is evaluated.

Microbial identification is often anything but a trivial exercise. Unless an isolate has been the subject of a taxonomic study, or is one of the cultures used to establish a commercial identification method database that is kept current, it is often difficult to ensure that an isolate belongs unequivocally to a specific taxon. In MRA, the risk assessor should evaluate the definition of the hazard and pay close attention to how different data sets used in the risk assessment to develop accurate exposure assessment and resulting risk characterization.

3.3 What Hazard Characteristics Can I Consider?

Risk assessors should consider several hazard characteristics when assessing a microorganism or its by-products (adapted from USACHPPM, 2009 and EPA 2009a):

a) **Infectivity** – the ability of a pathogen to enter, survive, and multiply (infect) in a host.

b) **Invasiveness** – the ability to degrade and migrate through the extracellular matrix and invade the host cells.

c) **Virulence** – the ability of the pathogen to defeat the host defenses; to increase the severity and longevity of the symptoms.

d) **Pathogenicity** – the ability to cause a disease state. Pathogenicity is the cumulative effect of virulence and invasiveness (see the glossary for a discussion of pathogenicity versus virulence).

e) **Host range** – which hosts a pathogen can infect. Some pathogens have very specific host ranges; therefore, the disease is limited to one host. Other pathogens have wide host ranges, and they can cause disease in many species.

f) **Horizontal gene transfer** - the movement (transfer) of genetic material (e.g., DNA) from one organism taxon to another that, with maintenance and expression of that genetic material, may lead to antibiotic resistance traits or acquisition of other virulence factors.

g) **Genetic drift –** random fluctuations in the frequency of alleles in a small isolated population, presumably owing to chance rather than natural selection.

h) **Replication** – the ability for a microorganism to multiply within the environment or the host.

i) **Persistence –** the ability of the microorganism to survive in the environment or the host.

j) **Transmissibility –** the ability of a microorganism to survive, replicate, and pass through animate or inanimate matrices and stay infective. In a broader context, the role of zoonosis or vectors may play a role in the spread or the pathway of the disease depending on the type of infectious agent.

k) **Opportunistic Pathogens** – the ability of a usually innocuous microorganism to cause an adverse health effect in a susceptible host.

l) **Secondary Transmission** – the spread of an infectious agent within a human population due to direct human-to-human contact between a primary case (infected or ill) and the secondary case who becomes infected or ill from that contact; or the secondary cases that arise from contact with fomites or contaminated food or water.

m) **Taxonomy and Strain –** the definition of the hazard with respect to traditional biological classification. 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. Some strains may not be infective for humans. In addition, the ratio of different strains in the environment can fluctuate.

n) **Resistance to control or treatment processes** – the ability of a pathogen to survive treatments, such as chlorination. If the risk assessment is for a performance target, then the treatment and control processes may be of central importance.

Genomics, proteomics, and metabonomics may all be important for hazard characterization as well as host characterization (EPA, 2006b).

3.4 How do Microbial Hazards Cause Adverse Outcomes?

The relationship between a host and a microorganism is complex with dynamic interplay; a pathogen can use a number of mechanisms to induce an illness. In general, these mechanisms include either direct invasion of the host cells and colonization of a specific tissue or organ, causing necrosis or other direct damage, triggering host responses that are self-damaging, or producing toxic by-products that can elicit an adverse effect through toxicological modes of action. Note microbes in the environment or the host may produce toxins. The conditions under which microbes produce toxins and how host exposure occurs is important to consider. For example hazardous algae blooms may result in oral or dermal exposure to toxins produced by algae in the environment, whereas, pathogenic *E. coli* produces toxins during infection which can continue to cause damage to the host even after the microbes die.

Among pathogenic organisms, several common patterns or themes in the cascade of events dictate the progression of disease. The first essential step in the establishment of a disease is the ability of the pathogen to adhere to a tissue. This step, while prompted by the pathogen, is often the result of a host-microorganism interaction that is host specific. The second step is the invasion/penetration of the host's epithelium, whether the skin, lining of the lungs, or the lining of the gastrointestinal tract. The pathogens are

able to invade the host cell and establish a niche where they can multiply. The success of a pathogen to initiate and cause disease is limited by its ability to counteract effectively the host defense mechanism and be able to multiply to a level that elicits a symptomatic response. The ability of the microorganism to defeat or evade the host defense response effectively determines the latency period, intensity, and persistence of the disease state.

Modeling mechanisms of infection (e.g., how molecular and cellular host and pathogen factors interact) may someday be applicable to MRA; however, currently the science is not developed enough for the pathogens of concern, and it is unclear how much value this feature would add given the large uncertainties in other areas of MRA.

3.4.1 What does Virulence and/or Pathogenicity Mean in the Context of Causing an Adverse Outcome?

Pathogenic microorganisms have virulence factors with specific modes of action for entry and colonization, and they produce adverse health effects.[9] The first step in assessing pathogenicity is to collect the microbial evidence for the adverse health effects associated with the agent of concern. To cause disease, pathogens must overcome various host defense systems, and their ability to do this is indicative of the virulence of the microorganism. Examples of some types of microbial virulence factors include:

a) factors that help the microorganism persist in the environment;

b) factors that help the microorganism evade the host immune system;

c) expression of surface proteins or polysaccharides that help bind the organism to a specific site in the host; and

d) production of toxins

In causing a disease, not only do the pathogenicity and virulence potentials of the microbial agent play a role, but also the degree of susceptibility of the host, the influence of environmental factors that determine exposure, and the level of the final outcome. Understanding the interactions between a microbial pathogen, the host, and the environment is key in determining the potential health impact a pathogen will have on an individual (or a population). The classic epidemiological triangle (disease triad) illustrates the inter-relationship between the host, pathogen, and environment (See Figure 1.2).

[9] Pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease. Virulence is the disease producing power of an organism, the degree of pathogenicity within a group or species.

3.5 What are the Mechanisms that May Lead to the Development of New Pathogens or Pathogens with New Traits?

Acquisition of new traits comes about by the transfer of genetic traits vertically or horizontally among microbial species. All living organisms have at least one natural mechanism for genetic transfer. Biotechnology takes advantage of these mechanisms to precisely transfer desired characteristics or remove undesirable ones in genetically modified bacteria. In prokaryotes, these mechanisms include: 1) conjugation, through which portions of genetic materials are exchanged between two related cells in physical contact; 2) transduction, which occurs through infection by a virus intermediate, a bacteriophage; and 3) transformation in which there is direct uptake and incorporation of extracellular DNA. Facilitating these transfers are genes for mobilization of DNA from one genomic compartment to another, as from a large replicon (chromosome) to a smaller one (plasmid). These are often found in insertion elements and transposons.

In the microbial world, mechanisms exist that consistently produce newer strains of pathogens or existing pathogens that acquire more virulent traits from other microorganisms. One such mechanism is the horizontal transfer of genes within and between viral and bacterial strains. While horizontal transfer of genes often results in reductions in fitness for (or in) the pathogen, the transfer results in more virulent and persistent viruses and other pathogens on some occasions. Recent advances in whole genome nucleotide sequence analysis demonstrate that viral, bacterial, and protozoan pathogen evolution includes horizontal gene transfer of virulence factors between different species and high taxa. Thus, an understanding of the role of horizontal gene transfer between different pathogens is essential for the evaluation of the possible introduction of new microbial hazards. This may result from an unintentional or deliberate environmental release of natural or genetically modified microorganisms.

It is commonly recognized that mobile genetic elements have contributed to rapid changes in virulence potential by facilitating the acquisition of new traits that increase pathogen survival, as well as adaptation in human hosts and in adverse environmental conditions. When mobilizing genomic elements (phages, plasmids, insertion elements, or transposons) acquire such functional gene segments, selection can segregate these into self-transmissible units, called ‘genomic islands’. Pathogenicity islands are units that contain specific traits or virulence factors that contribute to pathogenicity (Knapp et al., 1986; Schmidt and Hensel, 2004). The advent of whole genome sequencing and other advances in molecular biology has allowed development of criteria for recognizing pathogenicity islands in microorganisms of interest (Guzman et al., 2008; Yoon et al., 2007; Dobrindt et al., 2004).[10] In some cases, where the suspect microorganism is known to be related to ones that have been sequenced, the use of sequence analyses can be employed to look for components of pathogenicity islands or virulence factors.[11] As indicated in section 3.4.1, bacterial pathogenicity determinants are generally grouped as virulence factors or mechanisms that include antibiotic resistance, pore-forming toxins,

[10] http://www.gem.re.kr/paidb/about_paidb.php
[11] http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/micr.html; http://www.genomesonline.org/; http://www.tigr.org; http://www.sanger.ac.uk/

superantigens (Schmidt and Hensel, 2004)[12] and even quorum sensing (Lerat and Moran, 2004). Thus, the knowledge of mechanisms that have the potential to result in microorganisms with new pathogenic traits may be of critical importance in conducting certain types of risk assessments.

3.6 What are the Major Categories of Microorganisms?

The major microbial categories that cause adverse outcomes to humans are bacteria, fungi, viruses, protozoan, and algae. There is an additional category for indeterminate agents where the vehicle or pathway is important but the specific microbial agent can be indeterminate (Table 3.1). Helminthes (tapeworms, roundworms) are also considered hazardous organisms, particularly if direct exposure to feces is possible. Although helminthes are multicellular parasites and not microorganisms, they are sometimes considered in conjunction with microbial pathogens because infectious stages are too small to be easily detected by the unaided eye.

An array of microorganisms and associated literature on pathogenic genera, species, subspecies, strain, subtypes, and taxonomic characterization remain outside the scope of this document. Depending on the specific requirement of an assessment, it is recommended that an assessor consult relevant literature and subject matter experts as needed. Under some circumstances, the hazard may not be identifiable, however, the human health effects may be distinct. Hazardous agents may be of indeterminate type but may still be clinically defined enough to facilitate risk assessment approaches.

Table 3.1 presents some of the major categories of microbial hazards in the context of MRAs (Alexopoulos et al., 1996; Peter, 1998; Labbe and Garcia, 2001). The broad categorization of microbial organisms describes how an agent in a given category causes disease in humans. The placement of hazardous organisms into broad categories is particularly important in retrospective assessments to narrow the focus of investigation based on documented history for the category in question.

Many resources provide an overall picture of pathogens in specific media. Batz et al. (2004) constructed a comprehensive list of pathogens for the Foodborne Illness Risk Ranking Model (FIRRM) analytical software tool using data generated by various federal agencies. Batz et al. (2011) also estimated the ten pathogen-food combinations with the greatest burden on public health. CDC periodically reports estimates of the incidence of foodborne illness (Mead et al., 1999; Scallan et al., 2011a,b) and reports on waterborne disease surveillance (CDC, 1993, 1996, 1998, 2000, 2002, 2004, 2006, 2008). An additional list of foodborne pathogenic organisms and toxins compiled by FDA commonly called the “Bad Bug Book” may also be useful (CFSAN, 2006). Craun et al. (2010) provides a review of waterborne pathogens. The American Society for Microbiology (ASM) publishes the Manual of Clinical Microbiology, which is a significant resource on pathogens (ASM, 2011).

[12] http://www.gem.re.kr/paidb/about_paidb.php

Table 3.1 Major Categories of Foodborne and Waterborne Microorganisms

Category	Features				
	Examples	**Morphological**	**Physiological**	**Genetic**	**Pathogenicity Adaptation Mechanisms**
Bacteria	*E. coli* O157:H7, *Shigella Salmonella, Campylobacter, Listeria, Legionella* Cyanobacteria, *Vibrio Francisella tularensis, Brucella suis*	Single-celled prokaryotes	Metabolically diverse, invasive, produce intra/extracellular toxins	No nucleus, double stranded DNA, presence of extra chromosomal DNA/plasmids, mutate frequently, horizontal gene transfer mechanisms	Some species form spores to withstand adverse conditions. Mutation and gene transfer, pathogenicity islands, and other genetic traits/ mechanisms lead to frequent strain variation, acquisition of enhanced virulence traits, and adaptation to new environments toxin production
Viruses	Noroviruses, Adenoviruses, Enterovirus, Hepatitis A	Acellular, most are enveloped with geometric structures	Metabolically inactive, obligatory parasitic, host dependent	Single or double stranded RNA or DNA, mutate rapidly in host	Frequent genetic drift, shift, and other genetic mechanisms may lead to changes in antigenic properties, host survival/adaptation, and result in more virulent variants/strains
Protozoa	*Toxoplasma gondii, Giardia* spp., *Cryptosporidium hominis, Cryptosporidium parvum, Naegleria* spp.	Single-celled Eukaryotes of the Protista display different morphologic structures and stages of infectivity	Host dependent parasites	Nucleus present, but not known to mutate as frequently as bacteria and viruses	Cysts and spores formed to withstand adverse conditions. Relatively stable genome, however, mutation and gene transfer may lead to strain variation, enhanced virulence, and adaptation to new environment
Fungi	*Aspergillus fumigatus, Penicillium, Candida, Aspergillus flavus*	Eukaryote, mostly multi-cellular and filamentous, pathogenic fungi are mostly unicellular (e.g., yeasts)	Metabolically diverse, invasive, produce mycotoxins	Nucleus present, sometimes presence of extra chromosomal DNA/plasmids	Spores
Algae Chlorophyta, Rhodophyta Dinoflagellata	*Pfiesteria piscicid*, "red tide" *Gambierdiscus toxicus (Ciguatera)*	Single-celled photosynthetic organisms, often dinoflagellates, Eukaryotes	Metabolically diverse highly complex life cycle, a few toxin producing	Nucleus present	Three typical forms are classified as amoeboid, flagellated, and encysted varieties
Indeterminate agent*	Can vary, unknown	Can vary, unknown	Can vary	Can vary, unknown	Can vary, unknown

* The vehicle exposure/pathway may be important as the agent is indeterminate

EPA's Water Quality Criteria program,[13] which addresses microbial contamination of the nation's waters under SDWA and the CWA, provides information on microbial methods, Health Advisories, Regulatory Support, and Criteria Documents. Health Advisories serve as informal technical guidance to assist federal, state, and local officials responsible for protecting public health when emergency spills or contamination situations occur. Criteria documents and guidance for drinking water contaminants provide information so preliminary decisions can be made as to whether the contaminant is a significant health threat via drinking water exposure and whether sufficient data exist to perform quantitative risk assessments.

EPA's Candidate Contaminant List of microbial organisms (CCL3)[14] is a list of chemical and microbial agents currently not subject to any regulation based on a contaminant's potential to occur in public water systems and the potential for public health concern. The list of chemical contaminants includes cyanotoxins produced and released by cyanobacteria ("blue-green algae").

3.7 What Methodological Approaches can be Used to Identify and Quantify Microorganisms?

A risk assessor should become familiar with laboratory approaches for identifying and quantifying the microorganism(s) of concern. Any datasets that required laboratory methods used in risk assessment require careful review of issues related to sensitivity, specificity, limit of detection, sampling method, and sample size. It is important to review the differences if any, in the methods employed for detection of the disease agent in food, water, or other environmental sources and under clinical settings. Depending on the methods used, the interpretations of data and inferences may vary. Extensive reviews of microbial methods are available (CFSAN, 2001; USDA 2008a; AOAC International 2007 and many other sources). However, techniques and methods change, so staying up to date on the current status of different methods is important.

Identifying an unknown microorganism is a two-step process requiring methods to characterize the traits of an organism and approaches to interpret the characterization data. Methods to generate characterization data range from traditional culture-based phenotypic and biochemical tests to the more recently developed molecular techniques, such as polymerase chain reaction (PCR). Approaches can reflect the evolutionary inheritance of traits (e.g., lineal decent), the intrinsic properties of the organism regardless of how they were acquired, or a combination of both.

Methods used for identification and quantification are often related to similar methods used for classification of microbes. However, the different purposes of classification and identification require separate considerations, even when using the same technology. Under the controlled conditions of a classification study, a particular methodology may be exquisite in its ability to distinguish among selected related isolates, but the same method may only provide an approximate identification when one

[13] http://www.epa.gov/waterscience/criteria/humanhealth/microbial/
[14] http://www.epa.gov/ogwdw000/ccl/ccl3.html#microbial

encounters an isolate outside the lab without the benefit of closely related isolates available for comparison.

While traditional culturing methods for identification and quantification of microbes are still the mainstay for fecal indicators, it is clear that this approach by itself does not allow for complete evaluation of microbial organisms beyond the genus and species level. In addition, it does not detect strains that may be active/infectious but non-culturable. The classical culture-based approach relies on culture of the organism in question, isolation in pure culture, and a study of the morphological, biochemical, physiological, and other traits. However, not all microorganisms are culturable (see viable-but-not-culturable discussion in section 3.8).

More sophisticated methods have the ability to discriminate between subtypes and also capture information on pathogenicity determinants of interest at the genotypic and phenotypic levels, either with isolated cultures or even in mixed enrichments. Some molecular methods used as screening steps capture the virulence potential of a specific microorganism or multiple microorganisms (profiles) without the isolation steps that are generally less sensitive and more time-consuming.

Quantifying pathogens in food and water is challenging because the levels that can cause illness can be below the limit of detection for methods. For water samples, techniques for concentrating the microorganisms from large volumes into smaller volumes are often needed to permit detection and quantification.

3.8 Are there Concerns Regarding Microbial Detection Methods?

Purpose of risk assessment and choice of the method(s)

Discrimination down to the smallest organizational level (i.e., strain) is not necessarily the objective of all identifications done for risk assessments. The risk assessor determines the information that is critical for completing the assessment before pursuing a specific level of identification. If the pathogenic potential is associated with all members of some higher order taxa, then identification to that higher taxonomic level may be all that is required for broad-based assessments. For example, knowledge that an isolate is a member of a species complex with many shared characteristics may be sufficient to permit assessment of potential for pathogenic effects, such as the *Mycobacterium avium* Complex (MAC). If evaluation of a specific incident is the purpose of an assessment, it is important to note that even the lowest levels of taxon (single nucleotide polymorphism [SNP] variant) are known to elicit significant adverse outcomes. For examples, an enterohemorrhagic type of *E. coli*, known as serotype O157:H7, can cause serious hemolytic uremic syndrome and even death. It is known that the degree of adverse outcomes seen with *E. coli* O157:H7 infections vary distinctly among different clades (group of SNP subtypes) (Manning et al., 2008). Thus, in retrospective assessments in particular you should pay attention to detailed information within a taxonomic subunit.

Assessments for broad categories of microorganisms may only require genus-level identifications. Culturing techniques that involve phenotypic analyses may suffice. However, for this level of identification, 16S rDNA analyses have become commonplace and are usually deemed adequate for prokaryotes. Morphological features are traditionally used for fungi and protozoa, but biochemical and molecular methods are beginning to be essential to avoid misidentifications. In some cases, clinical specimens from human cases may have more specific typing information, whereas environmental samples may be evaluated with methods that detect and quantify a different subtype, or a broader group. The MRA documentation should include information on what level of pathogen characterization is relevant for each data set used. For example, if dose-response data are from one isolate (e.g., human trials with specific isolates of *Cryptosporidium*) and environmental occurrence data includes a broader set (e.g., *Cryptosporidium* counted by microscopy), the limitations of assumptions that are made when both these types of data are used in the same risk assessment should be transparently discussed.

Culture related issues

Many non-spore-forming bacteria exposed to environmental stress conditions may decline in number and not be detectable by traditional culture-based laboratory methods, depending on the level of detection for each method and the number of replicate cultures employed. To ensure that microorganisms in the VBNC state are not missed, be aware of relevant methods of direct identification such as molecular methodologies[15] and direct microscopy for detection. Microorganisms present at low levels may require specific enrichment cultures that allow growth of very low population numbers to levels high enough for traditional culture techniques. A VBNC state has been described where metabolic characteristics are quantifiable though cells cannot easily be grown on traditional culture media, most notably in the genus *Vibrio*, under conditions when water temperatures drop below 10°C (Smith and Oliver, 2006; Fischer-Le Saux et al., 2002; Rowman, 2004; Huq et al., 2000). Researchers have observed induction of VBNC state in many other pathogens, including *Listeria* and *E. coli* O157:H7. Dinu and Bach (2011) demonstrated a VBNC state for *E. coli* O157:H7 in a phyllosphere environment. Others have shown that under such stress conditions very small numbers of surviving cells are able to grow to higher levels using dead cell materials remaining in the stressed culture, therefore giving the appearance that the cell state had changed from nonculturable back to culturable (Bogosian et al., 1998). In addition, disinfectants and processing (e.g., heat) can also stress microbes and may be a factor to consider when processes are included in the risk assessment scenario. Molecular methods generally detect living and non-living pathogens, unless specific methods have been developed that target aspects of their growth viability (Sen and Ashbolt, 2011).

[15] There are methods to isolate the DNA from environmental samples that do not require a pre-enrichment culture, such as length heterogeneity-polymerase chain reaction (LH-PCR). Methods such as LH-PCR require as little as 10 ng of DNA in the PCR reaction to amplify 16S rRNA sequences (Bisson, et al., 2007). In addition, PCR has been used to detect antibiotic resistance genes in bacteria found in deli-meats (Li and Wang, 2010).

In the case of parasites and viruses that are nonculturable and do not replicate outside the host, serological or advanced molecular methods have been routinely utilized in identification and reporting. Other methods of detection may also be needed where pre-formed metabolites/toxins may be present, but the suspect pathogen cannot be recovered; for example, *Bacillus cereus* emetic toxin or endotoxins in aerosols.

Amplification of organisms by growing in culture can also exert selective pressure which can skew identification of microbes from environmental or clinical samples. Any methods related considerations might impact the final judgment of the relevance or quality of a particular study.

Level of discriminating power

The two major subtyping approaches commonly used are based on our ability to discriminate phenotypic or genotypic traits. The phenotypic approach includes serotyping, phage typing, multilocus enzyme electrophoresis, and esterase typing. The genetic subtyping approach encompasses pulsed field gel electrophoresis (PFGE), ribotyping, PCR-based subtyping techniques (e.g., random amplification of polymorphic DNA [RAPD]), amplified fragment length polymorphism (AFLP), PCR-restriction fragment length polymorphism (PCR-RFLP), repetitive element PCR (REP-PCR), DNA sequencing-based subtyping techniques (e.g., multilocus sequence typing [MLST]) (Liu, 2006), multilocus genotype typing (MLGT), and SNPs. Some of these molecular based subtyping techniques provide not only powerful discriminating capabilities to further identify a unit of potential hazard in question, but also to relate microbial traits to public health outcomes and, perhaps most importantly, to enable subtype-based surveillance to detect outbreaks.

Depending on the pathogen/unit of hazard of interest, pay close attention to the discriminating power of the methods used and level of details needed in risk assessment based on the risk management needs. For example, there are about 3,000 serotypes within the species *Salmonella enterica* with differing levels of human pathogenicity. Subtyping strategies include serotyping and further determination of the virulence traits important to public health. For example, subtyping *S. enterica* serotype Typhimurium definitive type 104 (DT104) involves identifying isolates through serotyping, followed by antimicrobial susceptibility testing to identify the R-type ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) antibiotic resistance pattern. *S. enterica* serotype Typhimurium isolates with R-type ACSSuT are subsequently phage typed to specifically identify DT104 (Akkina et al., 1999).

Some strains of the bacterial human pathogen *L. monocytogenes*, which consists of a range of strains/genotypes with varying degrees of pathogenicity, may be highly pathogenic and sometimes deadly; others may be relatively less virulent and cause little harm in the host. The ability to differentiate strains of *L. monocytogenes* is particularly important for microbial hazard identification and for tracking transmission of pathogenic strains within a given food processing environment for example. The purpose of subtyping is to link human infections that may be related, detect unusual clusters of

human disease, and determine the source of exposure thorough epidemiological investigation, as well as to find and control the source of contamination. The application of molecular techniques has facilitated the identification and characterization of major virulence-associated genes and proteins in *L. monocytogenes*. Various DNA fragment-based typing methods have been used to differentiate *L. monocytogenes* strains at the subspecies level to include epidemic clones, genotypes, lineage types, and serotypes.

Differences in methodological approaches and choice of method(s)

Phenotypic methods include techniques that directly or indirectly detect, measure, or characterize features of a microorganism resulting from the observable expression of (total) genetic constitution. Phenotypic characteristics of bacteria include morphological, physiological, and biochemical features. Methods for characterizing phenotype require growth of the microorganism in pure culture under appropriate conditions. Chemotaxonomic methods examine phenotype by using quantitative analysis of the organism's chemical constituents. Genotypic methods directly compare nucleic acid sequences rather than rely on gene expression.

Approaches to data interpretation can be determinative, numeric taxonomic, or phylogenetic. Sometimes neither genotypic nor phenotypic methods alone suffice for either classification or identification of some bacteria, but it may be possible to combine these methods using polyphasic taxonomy in which data from phenotypic, chemotaxonomic (e.g., cell-envelope lipids, electrophoresis patterns), and molecular methods (e.g., ribosomal deoxyribonucleic acid [rDNA], DNA gyrase, subunit B [gyrB]). When results from these methods all agree, you can usually rely on the outcome. However, conclusive data may not be always available for a microorganism of interest.

Other considerations for identification

The proper use of methodology needed to identify (classify) the subject of an assessment depends on the level of detail required, whether prospective or retrospective, and cannot be over emphasized. Use the historical perspective and the current state of taxonomy and nomenclature when the hazard units are whole taxa to ensure that relevant data and information are compiled. Scientific and technological advances make the field of microbial classification dynamic in that taxonomic and other classification methods are continually improving the field's understanding of the unit of infectious structure (e.g., genus, species, subtypes, SNPs), their risk potential, and mechanisms of pathogenicity. Phylogenetic analyses of microorganisms have resulted in frequent re-assignment of microorganisms into different genera or species, which may require tracking of microorganism synonyms. Additionally, the evolving nature of microorganisms enables them to acquire newer traits for pathogenicity, host range, specificity, adaptability, and survivability outside of and within the host that add to the complexity of HI.

You should consider defining the unit of hazard in the context of the evolving taxonomic information and risk management needs. For example, the term *Burkholderia*

cepacia had been applied both to a single species and groups of strains, termed genomovars (Mahenthiralingam, 2000; Vandamme et al., 1997, 2000). These groups of strains have subsequently acquired species status, with independent names being established. The latter have been called the *Burkholderia cepacia* complex (Bcc). *Burkholderia cepacia* is now construed as a single species within the Bcc, but isolates of other species in the complex have, at earlier times, been called *B. cepacia*, with attendant literature using only that epithet. However, at times the unit of hazard may not be identifiable as a structural taxonomic unit. Be aware of such issues to refine the scope of the unit of hazard in question.

When performing assessments with a hazard unit that is an individual strain or isolate, identification rather than taxonomy or nomenclature becomes the issue. In this case, identification refers to the verification of the labeling of an isolate, as well as the placement of an isolate within an existing taxon or the determination that it does not match any existing taxon. The purpose of identification ensures that the organism/unit of hazard is known and applies to a data set. For example, if occurrence data are for a set of isolates and dose-response data are for only one of the isolates, then discuss the uncertainty this information introduces.

In rare situations, the agent (e.g., genus, species, subtypes) may not be identifiable. However, defined host symptomatology may lead to an underlying suspect agent (Soller et al., 2010). Under such circumstances, the risk assessor may consider the vehicle of transmission of the suspect agent as the unit of hazard. Overall, the level of details in microbial HI/HC depends on the risk assessment characteristics and risk management needs (e.g., issues, goals).

3.9 What Host Factors Can I Take into Consideration?

The following factors are used to evaluate potential health effects due to exposure to a pathogen. Following each factor is a short description of how the factor may influence the health outcome. There may be little or no data for these factors, so their consideration may be limited to a qualitative discussion (adapted from USACHPPM, 2009; EPA 2009a). Some of the factors listed below are sometimes the defining characteristic of populations addressed in the risk assessment.

a) **Age/Life Stage –** Life stage refers to a distinguishable time frame in an individual's life characterized by unique and relatively stable behavioral and/or physiological characteristics that are associated with development and growth. Children and elderly are usually considered more susceptible due to immaturity or other potential weaknesses in their immune systems and diminished capacity to recuperate. Behaviors that affect pathogen exposure patterns may also be related to age. For example, children may also experience greater exposure (therefore larger doses) due to their behaviors (e.g., close-proximity playing, hand-to-mouth tendencies). The fact that all people pass through infancy and many through pregnancy and old age means that all people are relatively “more susceptible” at one time or another.

b) **Pregnancy** – Pregnant women and fetuses are considered to be a sensitive and perhaps a more susceptible life stage. 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). The underlying reason for increased susceptibility is due to the influence of pregnancy on the immune system. Pregnancy can also change exposure patterns, for example, water consumption in pregnant women is higher than the in the general population.

c) **Immune Status** – The immune system plays an important role in clearing pathogens from the human body, which influences potential health effects. Previous exposure may confer limited and/or short-term protective immunity (Frost et al., 2005) or long lasting immunity (especially to viruses). Conversely, infection and illness rates can be higher than would otherwise be anticipated for individuals or populations that have not previously been exposed to particular pathogens. "Traveler's diarrhea" is an observed phenomenon that exemplifies this type of situation. In addition, individuals with compromised immune systems who come into contact with pathogens may react very differently from individuals with intact immune systems. Definitions of populations or life stages 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. Note that children, newborns, the elderly, and pregnant women are also immunologically different from healthy adults.

d) **Natural Microbiota** – The presence of natural microbiota provide competition that influences the impact a pathogenic organism will exert on a host. Prior treatment with antimicrobials that alter the gut microbiome (the totality of microbes, their genetic elements (genomes), and environmental interactions) is a recognized risk factor for infection with *C. difficile* and *Salmonella*.

e) **Nutrition** – The nutritional state of the host affects the immune system. Malnourished individuals tend to have weaker immune defenses than well nourished individuals.

f) **Clearance Mechanisms**[16] – The human body has clearance systems to remove foreign particles from tissues. For example, nasal and oral clearance systems can remove airborne hazards and intestinal clearance mechanisms to remove gastrointestinal pathogens. Intestinal hypermotility may represent a host defense mechanism against *Giardia* (Anderson et al., 2006). Gastric acidity is a barrier that is a primary factor affecting the outcome of infections from food and waterborne pathogens. Certain behaviors, for example smoking, may affect the functionality of clearance mechanisms (e.g., mucociliary escalator); therefore, the functionality of clearance mechanisms of various host populations should be considered.

g) **Genetic Factors** – The expression of certain genetic factors may increase an individual's sensitivity to particular pathogens. Therefore, if genetic factors are known for the organism being assessed, expression level differences could be

[16] These may also be referred to as innate immunity.

considered when characterizing the pathogen and performing dose-response modeling. Some genetic factors that influence pathogen dynamics may be linked to race, which could be considered in the characterization of a hazard and dose-response modeling.

h) **Preexisting Conditions** – Preexisting conditions may affect a host's response to a pathogen and should be considered, if possible. Physical and emotional stressors may also influence host susceptibility.

i) **Carrier Status (Persistence in Population)** – The possibility of some humans to serve as "carriers" for pathogens needs to be considered when estimating the potential spread of pathogens, especially when the carrier may interact with hosts who are considered susceptible.

j) **Treatment Efficacy** – Whether or not effective treatment is available may be important to risk assessment. Treatment efficacy can be a major determinant of mortality.

k) **Social and Behavioral Traits** – 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, 2005; see age and behavior above). Social and behavioral traits may also be associated with cultural and racial identities and may be important for specific consideration in a risk assessment.

l) **Secondary Transmission** – Includes the spread of infection through direct human-to-human contact, fomites, and contaminated food or water.

3.10 How does Life Stage Affect Sensitivity to Infection and Disease Manifestation?

Sensitivity to infection is based on both exposure to a pathogen and the integrity of the immune system. Early life stages have a combination of factors that increase both the possibility of infection and intensity and duration of the disease. Young children spend the first two years or more close to the ground whether crawling or playing. Reliance on hands to move around, hand-to-mouth activity, and eating with hands as opposed to using utensils all raise the possibility for exposure and ingestion of pathogenic microorganisms compared with more mature individuals.

A second issue with children is the immature development of their immune systems. Infants have not had the exposure to the wide range of microbial stressors needed to afford protection. Newborn infants have passively acquired immunity from their mothers, which dissipates over the ensuing months. The passively acquired immunity provides some protection for newborn children, allowing them to develop the array of acquired immunity needed for full protection. Nonetheless, young children tend to be more susceptible than older individuals.

A significant consideration is the intensity and persistence of the disease in newborn infants. In the absence of prior experience with a specific pathogen, the body

requires more time to develop and process its immune response. Therefore, a pathogen can elicit more pronounced and longer lasting effects from infection resulting in more adverse outcomes in children compared with adults. In developing countries where treatments are limited, children succumb to gastrointestinal infections at a higher rate than in countries where timely medical intervention is widely available.

EPA's Risk Assessment Forum published *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants* (EPA, 2005a). Based on physiological and behavioral milestones, EPA recommends that children be grouped by the following ages:

a) Less than 12 months old: birth to <1 month, 1 to <3 months, 3 to <6 months, and 6 to <12 months.

b) Greater than 12 months old: 1 to <2 years, 2 to <3 years, 3 to <6 years, 6 to <11 years, 11 to <16 years, and 16 to <21 years.

In addition to children, the elderly may also be more susceptible to infections than healthy adults. Risk assessments designed to include the whole population should include discussion of the elderly, even if data specific to this subgroup are not available.

3.11 What Environmental Factors Can I Take into Consideration?

From the point of origin until it reaches the host, a given microorganism interacts with the environment at various levels that influence its survivability and virulence. Hence, at the point when the microorganism finally encounters the host, the ability to cause adverse outcomes depends on the complex interactions (microorganism-matrix, matrix-matrix, microorganism-carriers/vectors) that the microorganism experienced during transport. For example, the survivability, virulence, transmission, and successful development of the diseases/adverse outcome for a given microorganism may be influenced by the run-off from animal husbandry or from wild animal habitats into water bodies that can reach the target host directly through exposure to water or indirectly through zoonotic transmission routes or contaminated foods. Thus, you should consider the potential role of the non-host environmental conditions in influencing changes in the properties of a microorganism to render it potentially hazardous. These may include matrices (air, water, soil, food), fomites, vectors, and carriers. As the physical, chemical, and biological traits of the matrix can influence the microorganism's virulence and survivability, a thorough characterization of the matrix becomes critical in a given risk assessment.

There are also long-term changes that influence microorganism dynamics and occurrence in the environment. For example, seasonal changes, climate change, habitat changes, and urban environments can all impact microbial dynamics. If the risk assessment is intended to cover global spread of a disease as well as routine environmental considerations, it may be necessary to use detailed genetic information related to pathogen virulence and pathogenicity in the HI/HC for mapping a specific

hazard over a very large geographic region. This approach ties the HI/HC component temporally to exposure assessment by using specific information about the hazard such as strain evolution, potential for disease severity, and spread due to various environmental conditions, such as climatic changes, transit systems, population distribution, other carriers, and zoonotic predisposition. Thus, with the advancement in geospatial mapping, information technology, and microbial genetics technologies, risk assessors can now map, model, and predict the changes and spread of a specific hazard on a global scale almost in real time. Some risk assessment scenarios may include the impacts of these long-term factors.

Environmental factors can be considered with the epidemiological triad in mind (Figure 1.1), which also provides context for segueing into exposure assessment (see Chapter 5).

3.12 Summary

Hazard identification and hazard characterization provide a *qualitative* examination of the hazard identified. The dose-response assessment examines the *quantitative* relationship between hazard and effect. Hazard is often defined as the stressor or agent capable of causing an adverse effect on the exposed individual(s). Terms such as “agent” and “stressor” may sometimes be used synonymously with hazard. The major microbial categories that cause adverse outcomes to humans are bacteria, fungi, viruses, protozoan, and algae. An additional category includes indeterminate agents where the vehicle or pathway is important; the specific microbial agent can be indeterminate. Details regarding the methods used to identify and quantify microbes in environmental samples and in clinical samples may be important to discuss in a risk assessment.

Hazard characteristics include invasiveness, virulence, pathogenicity, host range, horizontal gene transfer, genetic drift, replication, persistence, transmissibility, secondary transmission, taxonomy and strain, and resistance to control or treatment processes.

Host factors include age/life, pregnancy, immune status, natural microbiota, nutrition, clearance mechanisms, genetic factors, preexisting conditions, carrier status (persistence in population), treatment (therapy), social and behavioral traits, and secondary transmission.

4. DOSE-RESPONSE ASSESSMENT

The "dose-response assessment" in MRA aims to establish the relationship between the dose of a pathogen that individuals or populations are exposed to and the probability of adverse health effects (e.g., infection, illness, death). Qualitative evaluation (hazard characterization) of a pathogen also is included in the conclusions drawn with regard to potential health impacts, particularly if data for a quantitative MRA or dose-response evaluation are not available. From the estimated quantitative relationship (dose-response model), the probability of potential adverse health effects of a given severity can be estimated from a given exposure to a pathogen. The exposure assessment and dose-response assessment are combined in the risk characterization step to describe the risk due to a particular exposure for a defined population and a defined hazard. It is important to note that because the dose-response assessment is used in the context of an exposure assessment, it is critical that the dose units used for quantitative estimates are comparable.[17]

No guidance is generally accepted regarding when the available microbial dose-response data are sufficiently representative for a particular scenario, as the factors associated with each scenario are likely to be unique to each case.

The information in this chapter is organized into two sections: 1) general considerations for dose-response modeling (section 4.1); and 2) current practice in dose-response modeling methods (section 4.2). Section 4.1 discusses the technical application of using statistical models to estimate health effects due to exposure. Section 4.2 focuses on technical aspects of current quantitative dose-response modeling.

4.1 What is Dose-Response Modeling and What are Some General Considerations for Dose-Response Modeling?

Dose-response modeling is the process of using mathematical relationships to describe the probability of an adverse health effect (e.g., infection, illness) occurring in an individual or the frequency of an adverse health effect in a population when that individual or population is exposed to a specific dose of pathogenic microorganisms (mathematical details are provided in section 4.2). The dose level may be measured in terms of a discrete number of organisms (e.g., oocysts), colony forming units (cfus), plaque forming units (pfus), or by molecular methods that may estimate gene copies or cell equivalents. A cfu represents one to several viable bacteria cells while a pfu represents one to several infectious viral particles. Alternatively, the model may specify a dose level by an average administered or ingested dose as well as represented by some other measure (e.g., median infectious dose [ID_{50}] units). The most common practice of dose-response modeling has been the fitting of limited data sets derived from experimental trials to statistical models that are often, but not always, biologically based. More recently, researchers also have been using outbreak data for dose-response

[17] For example, the number of organisms ingested in a serving for a human volunteer trial might be converted to number of organisms in a daily exposure. The units might change from event based in the dose-response assessment to a series of daily exposures over a lifetime for the exposure assessment.

modeling of disease incidence in populations (Teunis et al., 2005, 2008a), and physiologically based models are being developed to begin to capture the biological complexity associated with dose response (Blaser and Kirschner, 1999, 2007).

4.1.1 How do I Choose Between Modeling a Discrete Dose Versus an Average Dose?

Pathogen doses are inherently discrete. While chemical doses are expressed in mass units (e.g., mg/kg), pathogen doses are usually expressed as counts of organisms (e.g., oocysts/liter) or an average dose (e.g., mean cfu per serving). Although a group exposure may be described in terms of average pathogen concentration, pathogens are distributed in a particular medium (water, air, food) such that each individual may not receive exactly the same number of organisms. If the concentration of pathogens is very low, some individuals could be exposed to zero pathogens while others could be exposed to one or more pathogens. In this type of situation, the heterogeneity of the pathogen distribution in the matrix can be very important.

While the average dose of pathogens is continuous and can take any value, the actual number of organisms that an individual may consume is a discrete quantity. In the context of a clinical feeding trial, the distribution of pathogens or cfu in the delivery matrix generally is assumed to be random but homogenous (i.e., with the same mean), with the probability of exposure to a discrete quantity of organisms or cfu (0, 1, 2, etc.) given by the Poisson distribution. Because distributional data are preferred in MRA over point estimates (OMB, 2007b), if you only have data on the average dose, you can incorporate some assumptions about the distribution (e.g., Poisson, extra-Poisson, mixture). Using *C. parvum* as an example, for an average dose of 0.5 oocysts per unit, most (60 percent) individuals would consume no oocysts, while 30 percent would consume a single oocyst and 10 percent would consume 2 or more oocysts. Drinking water exposures are usually low, often below an average dose of 1×10^{-4} organisms per liter, which essentially means that 1 out of 10,000 individuals would ingest a single organism after drinking one liter of water at that concentration.[18] In modeling feeding trial data where administered doses are dilutions of a stock solution and not directly counted, it is reasonable to assume a Poisson distribution, unless there is reason to expect clumping of the organisms.[19] The assumption of a Poisson distribution may not be reasonable for modeling outbreak data, but may not make a difference given the high doses usually associated with outbreaks.

For low exposures, the discrete nature of the exposure constrains the maximum risk of infection to the probability of exposure. If the average dose of 1×10^{-4} and the pathogen were 100 percent infective, only one individual out of 10,000 exposed would

[18] In all practicality, doses less than 0.01 represent dilutions of single organisms, with an insignificant probability (though still not zero) of exposure to more than one organism. As a result the response is a virtually-linear function of dose at very low doses. Any function used for pathogen dose-response must follow the same "rule." Otherwise, a probability of infection greater than the probability of exposure could be predicted.

[19] Pathogens may clump together, particularly if the matrix has other components that aid clumping.

become infected on average. In this scenario, use discrete dose-response models because the risk is usually expressed as a unit-pathogen infectivity, which is greatly influenced by the Poisson variability in the dose. In contrast, the exposure distribution may be extremely skewed with some frequency of exposures in the range of millions to billions of organisms per exposure due to the potential for pathogen growth in some foods prior to consumption. In these cases, the Poisson variability around the mean dose is trivial because doses in this range are continuous and strict adherence to discrete models is not necessary. In these cases, you can use quantitative dose-response models based on continuous measures of pathogen dose to estimate the probability of infection or illness (Haas et al., 1999).

The Beta-Binomial dose-response model assumes that the exact number of organisms ingested is known, which is suitable for a feeding trial in which each administered dose has been enumerated. The exponential and beta-Poisson models assume that the number of organisms between subjects in a dose group is Poisson distributed with a fixed mean (FAO/WHO, 2009). If there is reason to believe that there is significant clumping of organisms, consider a more skewed distribution (see discussion in section 4.1.3).

4.1.2 What is the Difference Between a Threshold and a Non-Threshold Model?

A threshold model incorporates the assumption that there is a “dose or exposure below which no deleterious effect is expected to occur” (EPA, 2011a). A non-threshold model assumes that, even with the dose of one microorganism, there is a nonzero probability, possibly very small, of infection and subsequent illness (FAO/WHO, 2003). The basis for the non-threshold model is the one-hit theory (Haas et al., 1999; see section 4.1.3 below). From a practical perspective, the presence of a pathogenic threshold cannot be experimentally or empirically determined (FAO/WHO, 2003). There could be situations where threshold infectious levels (as measured by “average” pathogen concentrations) might exist. More data from ongoing studies is needed for additional insights on the possible existence of practical or actual thresholds. It has been suggested that mathematical models should be preferentially utilized that do not exhibit a threshold, but have sufficient inherent flexibility to allow high or low curvature at low-doses allowing for the mimicking of a “threshold-like” or sublinear response (FAO/WHO, 2003). However, a full range of models (e.g., threshold, non-threshold) can be considered to avoid the extremes of threshold versus non-threshold model assumptions (Coleman and Marks, 2000).

4.1.3 What is the One-Hit Model and When is it the Preferred Model?

One-hit (or no-threshold) dose-response models are generally the most relevant for foodborne and waterborne microbial dose-response assessment (Haas et al., 1999; FAO/WHO, 2003; NRC, 2003, 2005). However, these models may not apply to all pathogens that cause illness by producing pre-formed toxins in food, and they may be inappropriate for modeling illness and mortality.

FAO/WHO (2003) observed that despite the traditional concept of a "minimal infectious dose" in the microbial literature, attempts to define the numerical value of a threshold level of pathogens that must be ingested in order for the microorganism to produce infection or disease have typically been unsuccessful. "An alternative hypothesis is that, due to the potential for microorganisms to multiply within the host, infection may result from the survival of a single, viable, infectious pathogenic organism ('single-hit concept'). This implies that, no matter how low the dose, there is always, at least in a mathematical sense, and possibly very small, a non-zero probability of infection and illness" (FAO/WHO, 2003). Similarly, NRC (2003) noted that unlike chemical risk assessment, "microbial dose-response assessment for infectious pathogens does not produce any concept analogous to the no observed adverse effect level (NOAEL), since a single microbial cell may (under the right circumstances) produce illness." Finally, NRC (2005) observed: "In assessing risks attributable to exposure to microorganisms, it has frequently been asserted that there exists a threshold (minimum infectious dose) below which there is no risk to the population. Such a concept is not consistent with the current understanding of microbiological risk assessment.... The no-risk concept originated from the fact that in trials (either animal or human), low doses of microorganisms often produced no adverse effects in exposed subjects.... [However,] all animal and human exposure data that have been subjected to dose-response analysis are consistent with models in which the dose intercept is zero and the value of the conditional dose-response relationship for one organism is non-zero." Practically, it is not possible to distinguish between a very low non-zero risk and a true threshold. Risk assessors must rely on concept and theory to establish the most relevant dose-response modeling approaches. Therefore, in the ensuing discussion, the one-hit dose-response models are given preference over alternative models typically used for chemical dose-response modeling (e.g., log-normal, log-logistic, Weibull).

The one-hit model assumes that one infectious organism has the potential to cause infection. When an individual organism is ingested, the probability that the organism defeats the host barriers and initiates an infection may be represented by a unit-infectivity measure. This measure is termed "*r*" by convention, as *r* is the rate constant in the most-commonly used exponential dose-response model. The exponential distribution is based on the assumption that each organism is capable of initiating an infection and behaves independently from other organisms within the host, leading to a binomial probability of infection. The assumptions of a Poisson pathogen distribution and a binomial probability of infection lead to a family of models referred to as one-hit models, where the name relates to the concept that only a single organism is necessary to cause infection. Different pathogen distributions can be assumed in addition to the Poisson. If there is reason to believe that the pathogens are not uniformly distributed in the exposure medium (e.g., clumped), a negative binomial or other skewed distribution can be used (Haas et al., 1999; Teunis et al., 2008b), although the numerical algorithms become more complicated. From a modeling perspective, the one-hit model means that there is a non-zero probability that any given organism will survive the host defenses to initiate an infection, no matter how low the probability may be. Unit-infectivity probabilities can range from 1×10^{-10} to greater than 0.1 (Teunis et al., 1996; Haas et al., 1999). Although, conceptually based on biologically plausible mechanisms, most of these models do not

rely on independently validated parameters describing the mechanisms that model the underlying physiological processes explicitly, but rely on fitting curves to empirically observed data. The potential for applying physiologically-based models is discussed in section 4.2.8.

The one-hit concept also applies to the production of illness in that, once an individual is infected, progression to illness can proceed without additional exposure. Replication within the host of that single pathogen from the initial exposure would eventually result in enough pathogens to produce illness symptoms. However, the standard one-hit dose-response models are typically used for assessing the risk of infection rather than illness (or mortality). Morbidity and mortality may be expressed in only a small fraction of infected individuals. Because morbidity is commonly assumed to be independent of dose (but conditional on infection), it is a common practice to apply a morbidity or mortality ratio to the risk of infection (Haas et al., 1999). Typically, an estimate of the fraction of the infected population that becomes ill is determined by multiplying the fraction infected by a fixed morbidity ratio, which is usually determined from infectivity and illness data. Mortality is estimated in the same fashion, usually applying a mortality ratio as a function of morbidity in a stepwise fashion. The probability of illness also can be modeled directly, as a dose-dependent function of infection, but the computational algorithms are much more complicated (Teunis et al., 2008b). As a special case ("quorum sensing"), illness may not occur until the number of organisms reaches a critical mass at which time they release the toxins resulting in illness. However, the increase in the number of organisms is a result of growth within the host rather than an increase in the exposure dose. The concept of density dependent quorum sensing is distinct from a threshold for administered dose, because of the possibility, however small, that a single ingested organism may survive the multiple barriers in the gut to become established and reproduce (FAO/WHO, 2003). 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 based on analysis of the infection process and interpretation of epidemiological data.

4.1.4 What Important Factors Can I Consider in Dose-Response Assessment?

Exposure Route

The route of exposure can have a significant bearing on the dose-response curve. As noted above, for infection to occur and result in a disease state, the organism must penetrate the host's defenses, the capabilities of which vary with tissue type. The routes of exposure can influence both the slope of the dose-response curve as well as the manifestation. For example, adenovirus can be highly infectious through an inhalation route of exposure, but it appears to be less infectious through ingestion. Therefore, matching the route of exposure with appropriate dose-response information is important. Discussion of the implications of using the available dose-response dataset for broader exposure scenarios is helpful.

In chemical risk assessment, route-to-route extrapolation of internal dose for systemic effects can be performed if adequate toxicokinetic data exist.[20] Although systemic involvement can be an important factor, some pathogens can only infect specific tissue types. Other pathogens can infect many different tissue types and have a different dose response and health endpoint for each different tissue. For example, *Cryptosporidium* usually infects the gastrointestinal tissues, but in immunocompromised patients, it can infect other tissues including lungs. The infection of non-gastrointestinal tissues results in a different set of symptoms (O'Donoghue, 1995). Usually the most obvious concern is where the route of exposure leads directly to an effect in the most susceptible tissues, such as diarrhea from ingestion, pneumonia from inhalation, or dermal lesions from skin contact. However, for pathogens that cause illness through release of toxins, the host tissue that is most susceptible to the toxin may be remote from the physical location of pathogen replication.

Exposure Medium

The nature of the exposure medium can influence the probability that an individual pathogen will survive host defenses and initiate an infection. For oral exposure, pathogens can be ingested in food or water; or infection may result from direct contact with fomites or infected individuals. The primary factor in this process is the initial line of defense against the pathogen—stomach acid and digestive enzymes. If the ingestion medium serves to raise the pH in the stomach content, the probability of pathogen survival is enhanced for many organisms, perhaps by orders of magnitude, depending on the extent of acid buffering. The nature of the food matrix, for example, with respect to food structure and fat content, can vary considerably in enhancing or limiting survival of pathogens in the food matrix and in the host gastrointestinal ecosystem (Ross, 2008). In addition, taking stomach-acid reducers for acid-reflux disease can provide protection for the pathogen (Cash et al. 1974). Ingestion of drinking water, on the other hand, offers no protection from stomach acid.

In the dose-response documentation, you should discuss possible effects of different matrices relevant to the assessment. You could compare the delivery matrix used in generating dose-response data from a human feeding study to the matrix being considered in the exposure scenario. For example, if the dose-response data are from feeding trials using a water matrix and the exposure scenario in the risk assessment is juice, then you should clearly describe the difference and elaborate on what the potential implications of those differences might mean for the risk assessment.

[20] Chemical risk assessors have defined many different aspects of dose, such as potential dose, applied dose, absorbed dose, internal dose, and delivered or biologically effective dose.

Pathogen

During hazard identification, the pathogen of concern is defined. The dose-response assessment examines whether or not the pathogen of concern matches the pathogen for which dose-response data is critical. This issue is important because substantial variability in virulence and infectivity has been shown for closely related pathogens. For example, the relative infectivity of the *Salmonella enterica* serotypes (Coleman and Marks, 2000; Soller et al., 2007) and *C. parvum* isolates (Haas et al, 1999; Messner et al, 2001; Teunis et al., 2002; Englehardt and Swartout, 2004) used during human dose-response challenge studies varied by several orders of magnitude. Even with dose-response data for one or a few isolates of a species, risk managers may be interested in the species as a whole (e.g., the *C. parvum* example above) or the genus (given that a number of *Cryptosporidium* spp. may infect humans)(Xiao, 2010). Isolates only represent a small fraction of the genetic diversity of the species that is likely to occur in nature. Discussion of the implications of using the available dose-response dataset for broader exposure scenarios is helpful. No general guidance is available regarding when the available dose-response data are sufficiently representative for a particular scenario, because each scenario is likely to be unique.

Data on the actual pathogen of concern are preferred over data on surrogate organisms.[21] In cases where data on the pathogen of concern are not available, data on surrogate organisms can be used if solid biological evidence, such as common virulence factors, can support that choice. The biological basis for the use of the surrogate must be clear (FAO/WHO, 2003).

Haas et al. (1999) provides a method for testing whether differences among strains are statistically significant. It also is important to determine whether the strains can be considered a representative sample from the pathogen of concern or an extreme case. In cases where the strain is representative, you may want to use a mixed model with random strain effects. In cases where the strain is an extreme example, a bounding approach can be used (Vose, 2008).

Host

During hazard identification you should discuss host factors that are relevant for the risk assessment scenario. Given the current state of knowledge, quantitative microbial dose-response assessment for humans requires some human pathogenicity data. When using dose-response data from human trials, the characteristics of the population in the trials should be compared to the population defined in the scope of the risk assessment. This approach should discuss the types of individuals that are explicitly represented in the human trials (e.g., healthy adults) and individuals that were excluded from the trials (e.g., children, elderly, immune compromised, and pregnant women). Describe the potential implications to the different populations or life stages, and point out any tools used to compensate for these differences, such as information from

[21] "Surrogate organism" synonyms include "index pathogen" and "reference pathogen."

epidemiological data obtained during outbreak events. Also discuss the likelihood that individuals in the trials may have had some immunity to the pathogen being evaluated.

Human data are generally preferable to animal data. However, animal studies and *in vitro* studies may provide useful information on host-pathogen interactions (FAO/WHO, 2003). The strengths and limitations of those data, within the context of the risk assessment scenario, should be clearly explained. Some pathogens have evolved a narrow host range, while others have evolved a broad host range. Many important pathogens are host-specific, but more than 60 percent of human pathogens have multiple domestic mammalian hosts (Cleaveland et al., 2001). Among pathogens of wild primates, only 10 percent of bacteria, 13 percent of viruses, and 28 percent of protozoa are host-specific, and more than 100 pathogens (including 19 bacteria and 30 viruses) infect both wild primates and humans (Pedersen et al., 2005). Viruses mutate rapidly and can "jump" to new host species more easily than bacteria or protozoa. On the other hand, protozoa can be much less discriminating in their choice of host species, often infecting humans after passing through wild and domesticated animal species. However, the disease manifestations can be quite different, implying that different pathogenic mechanisms are in operation. As an example, *C. parvum* typically causes diarrhea in humans with no systemic involvement but kills (immunocompromised) mice from generalized systemic distribution with no diarrheal symptoms. One host cannot be used as a surrogate for another in all respects, and extrapolation from surrogate host data may require adjustments. Therefore, when surrogate animal models are used, the biological basis for and limitations of the use of the surrogate must be clear (FAO/WHO 2003).

<u>Endpoint</u>

The endpoints that are typically modeled in MRA are infection, illness, and death. Infection is the most immediate health effect for direct modeling because it is the first manifestation of exposure to a pathogen. However, infection is difficult to assess in humans if there are no clinical symptoms. Infection is generally equated with colonization of some tissue, either externally or internally. However, there can be uncertainty in correctly classifying infection. For example, a challenged volunteer may exhibit symptoms but no signs of infection (detection of pathogen in stool or seroconversion). There can also be a range in symptoms and definitions of "illness" may vary between studies.

Animal studies can include specific tissue analysis of sacrificed groups at intervals following the original inoculation to analyze the clearance time and/or the level of colonization (EPA, 2007a). However, colonization *per se* is difficult to measure in humans, so typical markers (measurement endpoints) of infection such as multiplication and shedding of the pathogen in feces or urine, presence of antibodies in the blood (seroconversion), or clinical symptoms are often used to determine if infection has occurred. Seroconversion is the change in pathogen-specific antibody levels between pre-challenge sera and post-challenge sera. The presence of pathogen-specific antibody in pre-challenge sera indicates prior infection with the pathogen, but not necessarily

infection with the same strain as that in the challenge inoculum. Detection of pathogen-specific antibody in pre-challenge sera does not necessarily indicate protective immunity.

Although (asymptomatic) infection has no direct adverse health impact, it can be crucial in determining the risk of illness and plays an important role when estimating the impact of secondary transmission. Illness and mortality are the primary endpoints of health concern. Illness endpoints could range from gastrointestinal distress to long-term sequelae. The risks of illness and death generally are estimated from the risk of infection by applying morbidity or mortality ratios, but can be modeled directly in certain circumstances. For example, the most commonly employed dose-response relationship for the ingestion of *Salmonella* through a waterborne route of exposure is for an endpoint of illness, not infection (Haas et al., 1999). Define the health endpoint of concern and specifically relate that definition to the clinical case definition used in any utilized dose-response studies. If there are any differences between the endpoint definition for the risk assessment and the case definition for data from trials or outbreak studies, you should describe the implications of those differences to the interpretation of the risk assessment results.

Inconsistencies in illness definitions can introduce uncertainties. For example, the definitions of diarrhea may differ slightly between different clinical trials or epidemiological studies and may be based on moisture context of stools. Illness definition endpoints can be quite complex. For example, EPA's bacterial ambient water quality criteria for recreational waters are based on highly credible gastrointestinal illness as defined as "any one of the following unmistakable or combinations of symptoms (within 8 to 10 days of swimming): (1) vomiting; (2) diarrhea with fever or a disabling condition (remained home, remained in bed or sought medical advice because of symptoms); (3) stomachache or nausea accompanied by a fever" (Dufour, 1984). Note that individuals with only diarrhea are not "counted" as a case with the above definition. Other studies may include diarrhea alone without fever as a valid case of gastrointestinal illness. For example, norovirus does not typically cause fever. You should pay close attention to the nuances of how endpoints are defined and discuss the impact any differences may have on the risk assessment.

How therapy can impact the health outcome should be discussed. Some infections are treatable and others are not. Therapy efficacy could influence determining the most relevant health outcome.

<u>Sources of Data</u>

Clearly document the sources of data considered, utilized, and omitted, and provide justification for those decisions. The direct pathogen-challenge studies produce the most precise human data (clinical feeding trials) where human volunteer subjects are fed known doses of a specific "enteric" pathogen and observed for gastro-intestinal symptoms over a certain period of time. In these challenge studies, clinical specimens (stool, vomitus, sera, saliva, PBMCs, etc.) are collected before the challenge and for days to weeks post-challenge. These specimens are used to determine infection status, pre-

challenge immune status and immune response to infection. These data can be modeled directly to obtain dose-response relationships and parameters for application to specific human exposure scenarios.

Prior to conducting a human dosing study or using data from an intentional human dosing study, one should consult the relevant ethics official or institutional review board (IRB) to ensure that the study is conducted in an appropriate manner or was conducted with sufficient protection of the subjects. For ethical reasons, direct pathogen-challenge studies are typically limited to healthy adult subjects challenged with a pathogen that has well-characterized health outcomes that are no more serious than temporary diarrhea that is self-clearing. Human pathogen-challenge data has been used in EPA regulations to predict the risk of giardiasis (EPA, 1989, 1998b, 1999) and cryptosporidiosis (EPA, 2006a). Data from clinical feeding trials are carefully collected and documented, but limitations with extrapolating from experimental conditions need to be addressed (e.g., subjects limited to healthy adults, use of non-wild-type pathogen strains, dose delivery matrix buffered to increase the likelihood of pathogen survival).

Human dose-response information can also be obtained from epidemiological data (primarily retrospective outbreak analyses). Although such data may be more representative of the actual host-pathogen-matrix combination uncertainty remains in the dose estimate, number of exposed or number of responders, or combinations of those variables. One significant advantage that epidemiological data have over clinical feeding trials is the potential for evaluating the relative risk of sensitive populations, as was done for *E. coli* O157:H7 (Teunis et al., 2004, 2008a). In this regard, epidemiological data can be useful to help generalize the available dose-response information to other population groups for whom specific dose-response information may not be available. These data also can provide information on the frequency of a range of outcomes for the general population and sensitive population subgroups, including infection, self-reported symptoms, medical visits, hospitalization, sequelae such as hemolytic uremic syndrome, and/or mortality.

Animal dose-response data have been used to estimate the human dose-response curve and to estimate the innate pathogen variability across strains. However, these data are difficult to translate directly to human dose response and require critical evaluation prior to use due to all of the uncertainties associated with interspecies extrapolation. Epidemiological information also can be used to calibrate ("anchor") dose-response curves derived from animal data with respect to the relevant human response range; FDA/USDA/CDC followed this process for the *L. monocytogenes* risk assessment by shifting the mouse mortality dose-response curve based on human mortality rates attributed to the pathogen (FDA/USDA/CDC, 2003). In addition, this approach used the variability of median lethal doses (LD_{50}s) in mice across *Listeria* strains to estimate the pathogen virulence variability. The approach assumes that the pathogen virulence variability was similar to the level of variability likely to be encountered in human exposures making it appropriate for dose-response uncertainty analysis. This use of animal data is not in lieu of human data, but is used to support assumptions about

characteristics of human data, such as potential range of variability. For more information on the use of animal data in MRA, see EPA (2009a).

For invasive bacteria and *Cryptosporidium*, the gnotobiotic piglet model has proven quite useful. It has been used to study dose-response effects for *Cryptosporidium, Campylobacter, Shigella*, rotavirus, *Helicobacter pylori, Salmonella* spp., and many *E. coli* types (enterotoxigenic; enteroaggregative; enterohemorrhagic such as O157:H7; enteropathogenic). Furthermore, infectious agents of swine – such as caliciviruses –are very similar to human caliciviruses and provide examples of animal-adapted pathogens (similar to the human) which could be used for dose-response experiments.

The rationale for the inclusion of other models, especially those from animal experimentation, is that they are informative as to the nature of the likely best models. The piglet model is also useful because of the similarities between the immune systems of swine and humans.

In general, *in vitro* models vary enormously as to their applicability; animal models vary less so, but still differ from humans. Some animal models may not be helpful with issues of infectivity or range/spectrum of syndromes, but the models could be useful in understanding the potential for adverse health effects in humans (Smith et al., 2008). *In vitro* experiments are often chosen to study mechanisms of entry rather than infectious dose. Cell lines may in fact be chosen because they are particularly permissive to the infectious agent in question.

Outbreak investigations often provide valuable information about the etiologic agent and unique opportunities for MRA data collection and model verification. A pathogen's ability to produce an outbreak depends on specific characteristics such as ability to survive in the environment, rate of growth or die off, potential to cause disease at a given dose, transmission route, and capacity to spread through person-to-person contact. Therefore, the details of outbreaks can help risk assessors develop exposure scenarios for specific pathogens, and data from outbreaks provide an important comparison for dose-response models based on human feeding trials or animal models. Outbreak investigation data have several important limitations. This approach focuses on identifying the vehicle rapidly to prevent additional infections so a narrow range of information is collected. Similarly, the exposure dose during an outbreak is often not quantified. Occasionally, data from concurrent drinking water testing are available, or frozen or unopened suspect food remains for sample collection and accurate identification and enumeration of pathogens. If actual levels of food or water contamination can be measured or estimated, it may be possible to estimate the dose-response relationship from outbreak data. 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).

Like outbreak data, annual surveillance statistics provide a way to evaluate MRA models. However, limitations with the accuracy and completeness of annual surveillance statistics can limit the usefulness of the surveillance data for evaluating or validating MRA models. The initial results of an MRA model can sometimes be cross-checked by comparison with public health surveillance data. The accuracy of dose-response models

may be assessed by combining them with exposure estimates known to be realistic and determining if the results approximate the incidence of illness estimated from surveillance data, taking into account the uncertainty due to under-reporting. 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. In addition, surveillance databases may have sufficient detail to analyze special populations or life stages, such as the elderly or the immunocompromised. With this information, the surveillance summaries or a series of reported foodborne or waterborne outbreaks also can identify the etiologic agents causing disease outbreaks and often the sources of the contamination. Consultation with public health surveillance experts can provide a "reality check" on the preliminary results of risk ranking and other quantitative MRAs.

4.1.5 How Can I Model the Spread of Disease in the Population?

In addition to primary transmission via contaminated media (e.g., water or food), many microbial pathogens can also be transmitted via person-to-person contact and cause infection and disease.[22] This route of microbial exposure, occurring from an infected person rather than from contaminated media, is also referred to as "secondary transmission." You can discuss any known features of secondary transmission when characterizing the microbial hazard, regardless of whether the scope of the risk assessment includes modeling secondary transmission. The ease of transmission of an organism from an infected individual to an individual that is susceptible to infection is an important consideration and should be evaluated during the exposure assessment. Refer to section 5.2.7 for a parallel discussion of this topic.

Secondary cases (often represented in epidemiological studies by a secondary attack rate) occur 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 distinguished from contamination of the immediate environment (e.g., toddlers sharing toys versus direct physical contact during play). Depending on the purpose of the assessment, the definition of secondary transmission may 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 result from person-to-environment-to-person transmission. Temporal and spatial limitations can be specifically noted in the definition of secondary transmission. You can discuss the full range of scenarios that qualify as both primary and secondary transmission. 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. The potential for reintroduction of the pathogen into the exposure media also could be within the definition of secondary transmission.

[22] Some situations blur the line between secondary transmission and environmental transmission. For example, infections due to a primary case causing an outbreak of cases in a daycare setting is usually classified as secondary transmission even though transmission may be from both direct human contacts and contaminated objects, food, or water exposures.

The degree of susceptibility to infection and illness is an important factor in deciding whether to explicitly model secondary transmission in a risk assessment. Population susceptibility and immunity change dynamically as a population is exposed to a pathogen. Many infections are asymptomatic; pathogens are commonly transmitted from person to person during asymptomatic infections or during asymptomatic periods of an infection (before or after symptoms) when infected individuals interact normally with susceptible individuals. A difference exists between immune response to infection (e.g., production of pathogen-specific serum antibodies) and development of protective immunity. For some pathogens, serum antibodies do not provide protection from subsequent infection. Pathogen-specific antibodies can be considered a marker of previous infection and of host susceptibility to the pathogen. Previous infections may increase the probability of illness in subsequent infections (e.g., Dengue fever). If a significant proportion of the population is immune to infection, such as from an immunization program, secondary spread of disease can be virtually prevented.

An attribute of an infectious agent that describes its ability to spread through a population is the basic reproduction rate or ratio of the infection (also called R_0). This ratio is estimated as the average number of secondary cases of an infection that occur in a completely susceptible population following introduction of a single case during its entire period of infectiousness (Rothman et al. 2008). R_0 reflects both the inherent infectiousness of a case of infection, and the factors that lead to transmission given infectiousness. If an infection has a value of R_0 less than 1, it cannot have sustained transmission in the population and will eventually go extinct; however isolated episodes and even brief chains of transmission may occur. If R_0 exceeds 1, the infection can spread in the population because each case is expected to cause more than one additional case, leading to an initially exponential growth in the number of cases (Tien and Earn, 2010). A larger basic reproduction rate produces an epidemic that is more difficult to control. Several factors that affect the basic reproduction rate of an infection include duration of infectivity of affected patients, the infectiousness of the organism, and the number of susceptible contacts in the population exposed during the infectious period. R_0 can be used in epidemiological transmission models to predict the trajectory of an epidemic (Rothman et al. 2008).

MRA models can be configured to account for secondary transmission and immunity in a population using a dynamic model (Anderson and May, 1991). These models, which can take several forms (deterministic or stochastic), characterize the dynamic epidemiological status of the population (e.g., susceptible to infection, symptomatic infection, immunity). Static MRA models do not, by their nature, consider secondary transmission, although dose-response parameters derived from static models may be incorporated into dynamic models. You can indicate if and how secondary transmission is included in the assessment. Inclusion of secondary transmission in MRAs often provides non-intuitive results (Eisenberg et al., 2008); therefore, if secondary transmission and other innate characteristics of infectious disease transmission are not included in the assessment, provide a sound justification for this decision. During planning and scoping, policy considerations may not introduce a concern about secondary

transmission; include a transparent discussion of how and whether secondary transmission was included in the model.

The use of these transmission models (discussed in section 6.5.2) in MRA has increased in the past 10–15 years with numerous model examples in the literature. For example, Zelner et al. (2010) used a transmission model to examine secondary spread through households after a point source foodborne outbreak. Eisenberg et al. (2005) used transmission models to analyze the 1993 *Cryptosporidium* drinking water outbreak focusing on: 1) disaggregating the risk associated with direct exposure to the contaminated water and subsequent secondary spread; 2) assessing the role that person-to-environment-to-person played in the outbreak; and 3) assessing the role that immunity played in the outbreak. Sheng et al. (2009) provided a framework for examining Environmental Infection Transmission Systems and motivated the use of dynamic MRA models. Eisenberg et al. (2002) provided a policy perspective for using transmission models in decision-making.

4.1.6 What Can I Address in Each Model to Improve Transparency?

To promote transparency, the dose-response assessment should address the following points for each dose-response model presented:

a) Assumptions

1) State the key model assumptions clearly.
2) Discuss assumptions inherent when extrapolating to doses lower than those used in studies.
3) Discuss flexibility in approaches to the dose-response relationship depending upon 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?).

b) Applicability of Models

1) Discuss the biological rationale for the model and logic for its selection.
2) Discuss the applicability of each model to various exposure situations.
3) Articulate strengths/weaknesses and advantages/disadvantages of the models.

c) Results

1) Discuss the type of information that the various models are expected to provide.
2) Discuss the use of likelihood methods to compare how well dose-response models fit the data.

4.2 What is Current Practice in Quantitative Dose-Response Modeling for Microbial Illness?

This section briefly summarizes some common dose-response models, and how those models have been used in previous MRAs. It also discusses the output of dose-response models and evaluates uncertainty and accounting for life stages and populations.

4.2.1 What Models Can I Use for Microbial Dose-Response Assessment?

Dose-response models are mathematical functions that yield a probability of an adverse health effect as a function of dose. Numerous dose-response relationships for microbial endpoints have been published in the peer-reviewed literature. Most of the dose-response relationships used to estimate adverse health effects in humans are based on either clinical feeding trials or outbreak data. As indicated in section 4.1.4, animal dose-response data have been used to estimate the human dose-response curve and to estimate the innate pathogen variability across strains. However, these data are difficult to translate directly to human dose response and require critical evaluation prior to use due to all of the uncertainties associated with interspecies extrapolation. No comprehensive summary of dose-response models is available for all human pathogens. However, a summary of peer-reviewed dose-response models for waterborne pathogens can be found in Table 4.1. Because this summary is for waterborne pathogens, the models presented are almost exclusively focused on the ingestion route of exposure, and in many cases were developed based on a relatively narrow population subgroup. The two most commonly used dose-response models are the exponential and beta-Poisson. Several alternative models also have been proposed as alternatives for MRA including two-parameter models (i.e., log-normal, log-logistic, and extreme value models) (Pinsky, 2000), three-parameter models (i.e.,Weibull gamma) (Farber et al., 1996), exponential gamma, Weibull exponential, the shifted Weibull model (Kodell, 2002), and neural network models (Fausett, 1994; Xie et al., 2000; Donahue, 2005). No single selection criterion for dose-response models is universally used. Section 4.2.3 provides several criteria that could be used. Always explain the logic behind the selected model, including the strengths and limitations of the model selection.

The models discussed in this section estimate risks for exposed individuals; thus, they are known as individual risk models.[23] Population risks (the incidence of disease among a group of exposed individuals) are generally constructed by combining the results of individual risk models with estimates of the distribution of doses in the exposed population (EPA, 2009a).

[23] Note that "individual" risk models may have as their outputs probability distributions of risk that can be interpreted to reflect (1) uncertainty in infectivity of the agent tested, and/or (2) variability in individual susceptibility among the experimental subjects.

Table 4.1 Overview of Dose-Response Relationships for Waterborne Pathogens[a] (Source: EPA 2009a; 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
C. jejuni[h,i]	Beta-Poisson	$\alpha = 0.145$ $\beta = 7.59$	Haas et al., 1999 Medema et al., 1996 Teunis et al., 1996
	Infection: Hypergeometric beta-Poisson Illness: Conditional on infection[g]	$\alpha = 0.024$ $\beta = 0.011$ $\eta = 3.63\times10^{-9}$ $r = 2.44\times10^{8}$	Teunis et al., 2005
Coxsackievirus	Exponential	$r = 0.0145$	Haas et al., 1999
Cryptosporidium	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	$\alpha = 0.060$ $\beta = 0.095$	Englehardt and Swartout, 2006
Echovirus 12	Exponential	$r = 0.0128$	Haas et al., 1999
	Beta-Poisson	$\alpha = 0.401$ $\beta = 227.2$	Teunis et al., 1996
		$\alpha = 0.374$ $\beta = 186.69$	Regli et al., 1991 Rose and Sobsey, 1993
		$\alpha = 1.3$ $\beta = 75$	Rose and Gerba, 1991
Entamoeba coli	Beta-Poisson	$\alpha = 0.1008$ $\beta = 0.3522$	Haas et al., 1999
E. coli (pathogenic strains)	Beta-Poisson	$\alpha = 0.1778$ $\beta = 1.78\times10^{6}$	Haas et al., 1999
E. coli O157:H7[j]	Beta-Poisson[e]	$\alpha = 0.248$ $\beta = 48.80$	Teunis et al., 2008a
	Hypergeometric beta-Poisson	$\alpha = 0.084$ $\beta = 1.44$ (children) $\alpha = 0.050$ $\beta = 1.001$ (adults)	Teunis et al., 2004
G. lamblia	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
Legionella	Exponential	$r = 0.06$	Armstrong and Haas, 2008
Norovirus	Infection (with aggregation): Hypergeometric function ${}_1F_1$ Illness: Conditional on Infection[g]	$\alpha = 0.040$ $\beta = 0.055$ $\eta = 2.55\times10^{-3}$ $r = 0.086$	Teunis et al., 2008b
Poliovirus I	Beta-Poisson	$\alpha = 0.1097$ $\beta = 1524$	Regli et al., 1991 Rose and Sobsey, 1993
		$\alpha = 15$ $\beta = 1000$	Rose and Gerba, 1991

Microorganism	Model	Parameters[b]	Reference(s)
	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., 1996
		$\alpha = 0.2531$ $\beta = 0.4265$	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
Salmonella	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
Salmonella (non-typhoid)	Beta-Poisson	$\alpha = 0.3126$ $\beta = 2884$	Haas et al., 1999
		$\alpha = 0.1324$ $\beta = 51.45$	FAO/WHO, 2002
Salmonella Typhi	Fractional polynomials	$\beta_1 = -18.1425$ $\beta_2 = 22.5300\times10^{-5}$	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
Shigella	Beta-Poisson	$\alpha = 0.21$ $\beta = 42.86$	Haas et al., 1999
V. cholerae	Beta-Poisson	$\alpha = 0.25$ $\beta = 16.2$	Haas et al., 1999

[a] These calculations 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 calculations can carry a substantial degree of uncertainty.

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

[c] Developed for inhalation exposure to adenovirus 4 aerosols.

[d] Estimated based on ID_{50} reported for the Texas A&M University (TAMU) isolate.

[e] Represents a meta-analysis of seven outbreaks and adjusted 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 × volume).

[h] An alternate dose-response model is proposed by Brynestad et al. (2008).

[i] Many of these models have been critiqued in the literature. For example, Coleman et al. (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.

[j] Cassin et al. (1998) used a Beta-Binomial model for *E. coli* O157 with the assumption that the virulence of the pathogen is similar to *Shigella dysenteriae*. The choice of parameter values for a and b are based on data from three published human feeding studies of two species *Shigella* (*S. dysenteriae and S. flexneri*). Powell et al. (2000) developed a dose-response model for illness by this organism that bounds the uncertainty in the dose-response relationship based on enteropathogenic *E. coli* (EPEC) and *Shigella dysenteriae*.

An overview of exponential, beta-Poisson, deterministic, and Bayesian hierarchical models is provided below and a summary of peer reviewed dose-response models is presented in Table 4.1.

The Exponential Model

The single-hit family of dose-response models was described previously (section 4.2.1) as the most relevant for microbial dose-response assessment. The simplest of the single-hit family of models is the exponential model (Equation 4.1).

$$p = 1 - e^{-r\mathrm{d}} \qquad \text{(Eq. 4.1)}$$

Where:

p is the cumulative probability of infection in the exposed population
d is the average pathogen dose in infectious units (organisms)
r is the probability of infection given ingestion of one organism

For the exponential model, r is a constant for the interaction of any given pathogen and host species. Each infectious particle within each host is assumed to have the same probability of survival. The host-pathogen interaction lacks inter-individual variability, so the exponential model assumes that the same probability of infection applies to every individual in the population. Despite this unrealistic-sounding assumption, the exponential model provides a good fit for a number of the human pathogen-challenge data sets (Teunis et al., 1996; Haas et al., 1999). The primary advantage of the exponential model is its computational simplicity. The primary disadvantage is that is does not account for inter-individual variability in the population.

The beta-Poisson Model

The primary limitation of the exponential model (no variability in r) is partially[24] overcome using the beta-Poisson model; this approach assigns a distribution to r to represent the variability in the pathogen-host interaction. The most common distribution applied to r is the beta, giving rise to the beta-Poisson model (more strictly, the beta-exponential). As r is a probability itself, the assigned distribution must have a range of 0 to 1. The beta distribution is the most flexible of such distributions, including shapes similar to Gaussian (normal), triangular, exponential, power law, uniform, and bimodal. The unit infectivity r-value is the mean of the distribution, which is readily calculated from the parameters. The two-parameter beta-Poisson does not have a fixed slope and is more flexible than the exponential. The beta-Poisson model still follows the rules (does not exceed the probability of exposure at low dose), but it is more biologically plausible than the exponential model and will fit better to data with higher variances. The beta-Poisson model is a function of the confluent hypergeometric function, which is a sum of an infinite number of terms and has no simple closed-form mathematical representation (Abromowitz and Stegun, 1964). Equation 4.2 provides the beta-Poisson cumulative probability.

[24] The distribution on r does not distinguish between pathogen and host variability.

$$1 - M(\alpha, \alpha+\beta, -d) \qquad \text{(Eq. 4.2)}$$

where M is the confluent hypergeometric function (the ${}_1F_1$ form), α and β are the beta distribution parameters, and d is the mean dose in pathogen infectious units. The solution is estimated numerically.

The Pareto II distribution, commonly called the beta-Poisson in the literature, was shown by Furumoto and Mickey (1967) to approximate the exact theoretical beta-Poisson model, and has found wide usage. The Pareto II distribution function for dose response is given by Equation 4.3.

$$1 - (1 + d/\beta)^{-\alpha} \qquad \text{(Eq. 4.3)}$$

where d is the dose, and α and β are parameters corresponding to the beta distribution parameters for specific ranges, the approximate form being valid for parameter values $\beta >> 1$ and $\alpha << \beta$ (Teunis and Havelaar, 2000). Outside of this range, the Pareto II can substantially overestimate the risk, sometimes predicting a probability of infection greater than the probability of exposure at low doses (Teunis and Havelaar, 2000). The term "beta-Poisson" will be used, henceforth, with reference to the exact form, while the analytic approximation will be referred to as the Pareto II. Both forms have been used for the gastro-enteric infection endpoint (Haas et al., 1996; Teunis and Havelaar, 1999; Englehardt and Swartout, 2004). Thus, the beta-Poisson is more flexible than the exponential model while retaining simplicity.

Accounting for Immunity

Whatever model is used, the risk of infection applies only to the susceptible population. In fitting the model to a particular human pathogen-challenge study data set, use available information to account for the fraction of immune individuals, sometimes even if the participants were prescreened for presence of antibodies to the pathogen. The general fitting algorithm for assessing the fraction of immune individuals is given in Equation 4.4.

$$(1 - fr) * F(d, \boldsymbol{\theta}) \qquad \text{(Eq. 4.4)}$$

where fr is the fraction of resistant (immune) individuals[25], F is the dose-response function (e.g., exponential, beta-Poisson), d is the dose, and $\boldsymbol{\theta}$ is the parameter vector associated with F. If the subjects were screened for prior exposure, offer an explanation of the (unexpected) finding of a resistant fraction when using this model. Explanations could be based on theoretical considerations or experimental conditions specific to the case.

[25] Alternatively, 1 – fr can be replaced by fs, the fraction of susceptible individuals.

Deterministic Models

The hit-theory models are stochastic in nature; each host may or may not become infected at any given pathogen dose. Moon et al. (2005) proposed deterministic models for microbial dose-response evaluation. These models assume that each host has a unique tolerance, or threshold dose, above which infection is 100 percent certain, similar to chemical dose-response assessment. These models are advantageous because they are more flexible than the hit-theory models and will tend to fit many data sets better. A disadvantage of this approach is that these models result in over prediction of risk at low doses (dilutions of single organisms) because they do not take into account the discrete nature of pathogen distribution. Over prediction is likely to happen for dose-response data sets characterized by high variability, high response at lower doses, or slowly increasing responses across large dose ranges. Over prediction is particularly prevalent in modeling uncertainty in bootstrap simulations. In addition, the biological plausibility of individual (deterministic) host thresholds has not been established for pathogens, as it has for chemicals. Therefore, deterministic models are not recommended, at least for low-dose extrapolation (e.g., determination of unit infectivity). However, deterministic models can be useful for high-dose risk estimation because of their ability to fit the response data better than the one-hit models.

Bayesian Hierarchical Models

Bayesian methods estimate dose-response model parameters and evaluate their uncertainty (Messner et al., 2001; Englehardt, 2004; Englehardt and Swartout, 2004). These methods are particularly useful in cases where data are available from multiple studies. One-stage or hierarchical models can be fit to the data using methods that include Markov Chain Monte Carlo Simulation (MCMC) (Gilks et al., 1996; EPA, 1997; FAO/WHO, 2003) (also refer to section 5.5 for more detail). A Markov chain is a stochastic model having discrete states in which the probability of being in any state at any time depends only on the state at the previous time and on the probability transition matrix. MCMC simulations can be used to generate samples from the joint posterior distribution (Messner et al., 2001). These models are advantageous because they are able to exploit subjective and related information in addition to numeric data.

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. The available knowledge, other than the theoretical form of the conditional distribution and empirical data already used for that purpose, is used to estimate the parameters of the distribution. 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 is determined by multiplying the posterior by the conditional dose-response function and integrating over the parameter space (Englehardt, 2004). Bootstrap methods (i.e., repetitive Monte Carlo sampling directly from the data or from data summary distributions) whether used in a Bayesian or frequentist framework, may

also be used to evaluate parameter uncertainty in dose-response models (Teunis et al., 1996; Haas et al., 1999; Englehardt and Swartout, 2006). These models can be more complicated than other models described here, are generally less familiar to scientists and managers, and can be difficult to explain.

Previous work used Bayesian hierarchical models to develop dose-response relationships for pathogens based on outbreak data rather than feeding study data. Teunis et al. (2005) analyzed *C. 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, a certain probability of illness (p0) 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 was developed, given that the individual is infected and had mean dose D. Non-informative prior distributions for the parameters were defined. 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 and the probability of illness given infection. Uncertainty intervals for these dose-response functions were computed by using MCMC to simulate vectors of parameter values. Teunis et al. (2008a) also analyzed data from eight outbreaks of *E. coli* O157:H7 using a hierarchical Bayes model; the researchers used Bayesian methods to analyze dose-response functions for the Norwalk virus, based on a volunteer study (Teunis et al., 2008b).

4.2.2 What is the Output of a Dose-Response Assessment?

The output of a dose-response assessment is a value or a set of values for the dose-response parameters. For example, for the exponential dose-response model (described in section 4.2.1), a single value ("r") would be required, and for dose-response models having more than one parameter, a set of parameter values would be required. For many of the most common dose-response functions, the relationship between exposure and risk is linear at low doses (Haas et al., 1999). For exposures to many organisms at once (such as in food), the risk of infection needs to be calculated from the mathematical dose-response function itself.

Computing the risk of infection may be necessary to determine the population at risk for illness, but infection, in itself, is not necessarily adverse. Therefore, information on the rate of illness, given infection, is needed to perform a meaningful risk assessment. Although orally ingested pathogens can cause a number of symptoms, some severe enough to be life-threatening, the only endpoints examined in human studies (for ethical reasons) are related to gastrointestinal illness, primarily diarrhea. The risk of illness is estimated in a similar fashion as for infection except that the dose-response model is constrained such that an illness response is strictly conditional on infection. The constraint is trivial for the one-parameter exponential model, as the slope is fixed. For models in which the slope can change (more than one parameter), a higher risk of illness

than infection can be predicted for some data sets if the constraints are not strict. Illness without infection is not biologically plausible so constraints are necessary to avoid this implausibility. Applying strict constraints in such models is not a trivial exercise and is a topic of ongoing research. The output of an illness dose-response assessment generally is a morbidity ratio, which is the fraction of those who are infected that become ill. A common practice is to assume that the risk of illness is constant once an individual becomes infected, no matter what the dose. In this case, the morbidity ratio is simply the number of ill individuals divided by the number of infected individuals. Dose-dependent morbidity ratios, where the conditional (on infection) risk of illness increases with increasing dose, are more difficult to model, requiring the strictly constrained model previously discussed (refer to Teunis et al., 2005). For drinking water risk assessments where the exposures are frequently very low, the occasionally large difference between constant and dose-dependent morbidity ratios can be highly significant.

Transparency in your discussion of the dose-response output is recommended. For example, the dose-response is based on a defined health endpoint, a defined human population (e.g., the population used in a clinical trial or an epidemiological study), and is influenced by model selection. Be clear about all the constraining features of the data and what assumptions were made when those data were used to extrapolate to broader human populations or health endpoints. For example, the risk managers who will use the risk assessment results to inform their decision making need to understand the implications of dose-response data based on healthy adults, when their goal is protection of the general population that includes all life stages and sensitive populations.

4.2.3 How do I Fit Models to Existing Dose-Response Data?

Table 4.1 provides a summary of many dose-response relationships published in the peer-reviewed literature for waterborne exposures. In many cases, the most appropriate dose-response relationship has already been peer reviewed. If an appropriate dose-response relationship for the specific pathogen/matrix combination is not available, it can be derived from a relationship in several ways. If an appropriate dose-response data set is available, try to fit the data to a mathematical dose-response model, which provides a prediction of the incidence of the effect in the population, given a specific exposure level or dose. The dose-dependent probability distribution is defined by a mathematical equation with one or more parameters. The best values for those parameters are determined by assessing the likelihood of observing the data, given specific parameter values. The parameter values that result in the greatest likelihood of the data are chosen as the most representative ones. The best parameter values can be determined directly on the data (as in a “frequentist” approach), or by updating a prior judgment as to what the parameters might be (a “Bayesian” approach). In either case, the resulting fitted dose-response model predicts human response, either infection or illness, to pathogen exposure for a specific risk-assessment scenario.

Frequentist Approach

The typical dose-response assessment is performed by a direct fit of the functional form to the data using a "frequentist" approach. The fitted parameters represent the frequency with which some event has happened previously. The fitted parameters, however, are generally used to predict the future occurrence of that event. Proponents of Bayesian methods argue that a Bayesian approach is a virtual requirement when trying to assess *probabilities* of future outcomes, rather than the *frequencies* of past events (Bernardo and Smith, 1994; Berry, 1996; Carlin and Louis, 2001). Bayesian methods provide a rigorous framework for common-sense interpretation of statistical conclusions. Frequentist approaches can only place confidence limits on a result that depends on specific conditions, leading to inferences that might be made in repeated practice. Bayesian proponents point out that most people erroneously interpret frequentist results in the Bayesian sense (Gelman et al., 2004). Bayesian methods generally provide much greater flexibility than do frequentist methods, especially with very complex, data-limited problems.

Bayesian Approach

Bayesian methods, however, are not without problems of their own. First, computation of the more complex integrals generally involves complex numerical techniques with which many practitioners will not be familiar. Most analysts will have to rely on programs, which, in themselves require some degree of mastery of functional coding techniques. Second, Bayes theorem requires that a prior relationship between dose and response be specified, which can be problematic for pathogens. The prior, which is usually subjective, can vary among investigators. Non-informative priors, generally uniform distributions over a wide range of parameter values, address the subjectivity issue but can make strong statements about prior belief of infectivity. An example of the latter and, perhaps, the only truly non-informative prior would be the simplest prior on the r parameter for the exponential distribution (see section 4.2.1) of a uniform distribution between 0 and 1 (the full range of r). Although this prior suggests limited knowledge *a priori* about infectivity, it establishes a prior expected value of 0.5 for r, which is much greater than any known actual pathogen infectivity. The Bayesian posterior is a compromise between the prior and the data, which requires a lot of data to move the answer towards a less extreme value.

Use of Bayesian techniques in the literature includes a number of analyses of *C. parvum* (Teunis and Havelaar, 1999; Messner et al., 2001; Englehardt and Swartout, 2004, 2006; EPA, 2006a). These analyses are largely hierarchical, assessing the aggregate infectivity of several isolates of *C. parvum* and treating each one as a distinct strain. The outputs of most of these analyses consist of distributions of uncertainty for "hyperparameters" of *C. parvum* infectivity across strains. EPA used this approach to support the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) in 2006 (EPA, 2006a). One of the analyses did not assume that the *C. parvum* isolates were different, evaluating the aggregate illness-response data across all studies (Englehardt and Swartout, 2006). This analysis and one other (Englehardt and Swartout, 2004) used an

unconditional Bayesian form, in which the posterior represented the dose-response function integrated over the entire range of parameter values. This unconditional or "predictive" form is independent of any pre-selected confidence level (e.g., 95 percent) and can be considered to be the expected value under uncertainty. All of the cited *C. parvum* assessments can be considered to be meta-analyses of sorts, as they incorporate data from more than one study into a single aggregate output. Meta-analysis generally allows for a weight-of-evidence approach, where a more objective review of data may occur. Although none of these analyses did so, individual data sets can be assigned specific weights (other than unity) prior to quantitative analysis in a meta-analysis. In this case, a Bayesian framework offers a rigorous approach to evaluate such weightings. Bayesian model averaging is a technique that formalizes this process (Hoeting et al., 1999).

4.2.4 How Can I Evaluate Uncertainty in Dose Response?

Uncertainty is imperfect knowledge. Uncertainty can be reduced by accumulating more information. Uncertainty can exist in several components of an MRA including the dose-response relationship. It is possible to characterize the precision with which the dose-response curve has been determined. The statistical confidence limits that are sometimes provided along with the dose-response parameters typically do not represent the full expression of uncertainty in the dose-response relationship. For single-hit models derived from dosing studies, uncertainty can be characterized by computing the confidence limits to the parameters of the dose-response curve and also the upper and lower envelope around the dose-response curve (Haas et al., 1999). A likelihood-based approach can be used for this purpose. This yields an m-dimensional region, where m is the number of parameters in the dose-response model under evaluation (one for the exponential, two for the beta Poisson, etc.). The computational details of this approach are beyond the scope of this document, but interested assessors are referred to Chapter 7 of Haas et al. (1999). However, most human challenge studies do not attempt to test very low doses of a pathogen because of sample size limitations. So the dose-response relationship at low doses (that may be most representative of contamination levels encountered in food and water) is extrapolated based on the type of model fit to the data in mid to higher doses.

In hierarchical Bayesian approaches, the output of the uncertainty analysis can be in the form of distributions on the model parameters or "hyperparameters," such as the mean of the fitted beta distribution (the average unit infectivity) for the beta-Poisson dose-response model. A distribution of plausible bounds on the response at any dose can then be generated to obtain, for example, 95% confidence limits on the response at a given dose.

In the predictive Bayesian models, the parameter uncertainty is integrated with the response variability to obtain a single dose-response curve without confidence bounds, but whose shape depends on the amount of information available; the shape becomes narrower (less uncertain) as the amount of information increases (Englehardt, 2004), generally resulting in a prediction of lower mean risk than when information is sparse.

Another technique is the parametric bootstrap analysis, in which the result of the initial dose-response model fit to the data is assumed to be the true response relationship. The responses at each dose are then regenerated repeatedly based on the respective fitted response probabilities assuming an underlying response distribution (generally binomial). A distribution of "resampled" responses is obtained for each dose, from which bootstrap confidence bounds on the entire dose-response curve can be calculated.

Regardless of the model used to characterize dose response, uncertainty in characterizing host susceptibility remains. Some hosts may have unknown genetic factors that make them resistant to a particular pathogen even at high doses (e.g., Norwalk virus, see Lindesmith et al., 2003). Furthermore, most human challenge studies have small numbers of subjects, with few doses tested and small numbers of subjects at each dose. Therefore, uncertainty about the classification of the infection status or illness status of a single volunteer may have a large impact on the results at a single dose level and may affect the accuracy of the dose-response model.

4.2.5 What is Variability in Dose Response?

Variability describes a range of possible events that result from chance; it can only be altered by changing the chance of something occurring. Variability also can exist in several components of an MRA including the dose-response parameter values. Dose response involves the interaction of host and pathogen, so any factors associated with either the host or the pathogen have variability and the interaction itself is also variable. The variability in factors presented in section 4.1.4 may be quantitative or qualitative in nature. The simplest approach to address dose-response variability is through stratification (i.e., assess variability between isolates and strains by examining multiple dosing studies looking at different strains). Statistical techniques may characterize variability depending on the needs of the particular risk assessment, such as:

a) Dose-response relationships to account for differences between isolates or strains, or to account for variability in the titer of dose given to individual subjects in a challenge study;

b) Host immune responses (both immunity and susceptibility);

c) Duration of host immune responses;

d) Host characteristics that influence the dose-response relationship (population differences);

e) Health effects.

4.2.6 How Can I Account for Life Stages and Different Populations in Dose-Response Models?

A life stage is something the whole population passes through, such as childhood. At any given moment, only part of the population is in childhood, but referring to children as a population underestimates the importance of childhood life stages in the larger picture. EPA breaks childhood down into ten age groups based on behavioral and physiological milestones (EPA, 2005a; refer to sections 3.9 and 5.2.3). Some life stages, such as children and the elderly, are more sensitive to pathogens because of host susceptibility and/or behavior patterns when compared to other life stages, such as adults. In chemical risk assessment, life stages and sensitive populations are frequently taken into account by the application of uncertainty factors. The use of uncertainty factors for pathogens, however, is somewhat problematic because of the discrete nature of exposure. Unlike for chemicals, pathogen dose is usually expressed in non-reducible organism-level units as an average, such that fractional doses represent probabilities of exposure to single organisms. When considering low-dose exposures, a dose of 10^{-4} for a chemical may be in units of milligrams or micrograms, representing perhaps 10^{18} molecules, while the same dose for a pathogen would represent a 1 in 10,000 chance of ingesting a single organism for a given exposure. Depending on relative infectivity of the pathogen, the indiscriminate application of a fixed uncertainty factor could result in an impossible risk (i.e., risk of infection greater than probability of exposure) or an arbitrarily high risk inconsistent with the overall exposed population. Accounting for sensitivity, life stages and populations require an estimate of the relative infectivity for that population subgroup compared to the general population, as well as the fraction of that population subgroup with respect to the entire population. With this information, a new population infectivity parameter could be calculated as a weighted average. For example, simply stating that you believe that a particular life stage is 10 times more susceptible than the healthy adult population represented by the experimental data is not adequate, in itself. In this case, if the r-value for the healthy adult population is greater than 0.1, an impossible risk (> 1) is projected for the life stage. Each case is likely to require a unique solution, with professional judgment playing a large role. For some pathogens, the probability of infection or disease for sensitive life stages and populations may not differ from the normal population, but the severity of the disease outcome may differ.

4.2.7 Can I Use Uncertainty, Modifying, or Adjustment Factors in a Microbial Dose-Response Assessment?

There are no standard guidelines for the application of uncertainty, modifying, or adjustment factors in MRA as there are in chemical risk assessment. In chemical risk assessments, uncertainty factors are usually applied as factors of 3 or 10 and are applied to effect levels derived empirically (e.g., no observed adverse effect level) to accommodate for a lack of knowledge associated with interspecies extrapolation, high- to low-dose extrapolation (i.e., effect to no-effect), population variation (i.e., protection of sensitive populations), and extrapolation across exposure durations (e.g., subchronic to chronic). The areas of uncertainty potentially most relevant to MRA are the interspecies, sensitive life stages, and sensitive population extrapolations. As an example for

interspecies extrapolation, sound allometric-scaling principles relates the uptake and whole-body distribution of chemicals and strongly conserved mechanisms of toxicity across mammalian species. No corresponding agreed upon principles for cross species pathogenicity relationships exist. Because many pathogens are highly species-specific or produce different effects in different species, and immune response mechanisms can be highly variable across species, use of uncertainty, modifying or adjustment factors to justify extrapolation is highly suspect.

For chemical risk assessment, all uncertainty factors are applied as a divisor of the dose to obtain a quasi-threshold exposure level. For MRA, the assessor would have to pay attention to the absolute value of the computed risks to prevent implausible or impossible risks (e.g., population risks near or greater than 100% when uncertainty factors are applied). Furthermore, to obtain an overall population risk (adjusted for sensitive life stages and different populations), the assessor would have to know the proportion of the population that is sensitive (for proper weighting of each population subgroup-specific risk). An example of this process can be found in a human population infection-response assessment for *C. parvum*, in which human pathogen-challenge study data were combined probabilistically with assumptions about the size of sensitive and resistant populations to obtain an estimate of overall population response (Englehardt and Swartout, 2004).

Because of the foregoing considerations, it is not usual practice in MRA to use uncertainty or modifying factors in a manner similar to their use in chemical risk assessment. An important distinction between uncertainty factors and adjustment factors is that uncertainty factors account for unknown distributions of sensitivity and adjustment factors account for known differences in response. Case-specific adjustment factors can be employed if strong defensible evidence supports their use. For example, the FDA/USDA/CDC risk assessment for *L. monocytogenes* in ready-to-eat foods used epidemiological data to justify the application of adjustment factors. Scaling factors were used to adjust mouse-derived dose-response curves to make the data applicable to humans (FDA/USDA/CDC, 2003). The size of the scaling factor was determined by surveillance data reported to FoodNet[26] for the populations modeled in the risk assessment. The dose-response curve in the FDA risk assessment on *V. parahaemolyticus* was adjusted to reflect CDC's illness estimate. The adjustment factor represents the effect of the apparent differences between the dose response observed in human volunteers under controlled conditions versus that in the general population when exposure is associated with the oyster food matrix.

4.2.8 Are Other Modeling Methods Being Developed?

Dose-response modeling is an active area of current research. For example, compartmental models are being developed to capture the biological complexity associated with dose response (Blaser and Kirschner, 1999, 2007; Serra et al., 2009; Mayer et al., 2011). These physiologic models begin with the development of conceptual models that break the process from exposure to establishment of infection to the

[26] http://www.cdc.gov/FoodNet/

expression of illness into compartments. These compartments serve as "steps" in the process, and the parameters inside each step are captured in the model. Published research and/or new research provide or will provide the values for each parameter. One of the strengths of the risk analysis concept is the clear identification of data needs, which can then be translated into research priorities. Dose-response models also are being developed to account for potential differences between primary and person-to-person transmission and to evaluate non-sigmoidal curves. The maturing of these and other modeling techniques could lead to decreased dose-response uncertainty.

4.3 Summary

The "dose-response assessment" component of an MRA establishes the relationship between the dose of a pathogen that individuals or populations are exposed to and the probability of adverse health effects (e.g., infection, illness, death) to individuals or populations. The exposure assessment and dose-response assessment are combined in the risk characterization step to describe risk due to a particular exposure for a defined population and a defined hazard.

Dose-response modeling is the process of using mathematical relationships to describe the probability of an adverse health effect (e.g., infection, illness) occurring in an individual or the frequency of an adverse health effect in a population when that individual or population is exposed to a specific dose of pathogenic microorganisms. The dose level may be measured in terms of a discrete number of organisms (e.g., oocysts), colony forming units (cfus), plaque forming units (pfus), or by molecular methods. The most common practice of dose-response modeling fits limited data sets that have been derived from experimental trials to statistical models that are often biologically based. More recently, researchers have used outbreak data for dose-response modeling of disease incidence in populations, and physiologically based models are being developed to begin to capture the biological complexity associated with dose response. One-hit (or no-threshold) dose-response models are generally the most relevant for foodborne and waterborne microbial dose-response assessment. The most commonly used dose-response models are the exponential and the Beta-Poisson. However, these models may not apply to all pathogens that cause illness by producing pre-formed toxins in food, and they may be inappropriate for modeling illness and mortality.

The route of exposure and the exposure medium can have a significant bearing on the dose-response relationship. Therefore, matching the route of exposure and the exposure medium with appropriate dose-response information is important. It is also important to transparently describe variability and uncertainty in dose-response relationships. A summary of many peer-reviewed dose-response models for waterborne pathogens can be found in Table 4.1.

5. EXPOSURE ASSESSMENT

The goal of exposure assessment in MRA is to determine the route, frequency, duration, and magnitude (amount) of exposure to a microbial hazard in a population.

Microbial agents may come from more than one source, may be transmitted via multiple routes of exposure, and may be spread via secondary transmission. Moreover, these routes of exposure may be inter-related. Exposure routes relevant to a given microbial hazard are situation-dependent and influenced by the inherent properties of the microorganism and its potential host(s). An exposure source can originate from either natural or anthropogenic events, activities, or locations that generate or release microbial hazards.

A number of factors define microbial exposure, including the sources and pathways of exposure, the growth and/or decline in numbers of microorganisms, and variable intake amounts among individuals. Often, some of the necessary data for a microbial exposure assessment are either lacking (i.e., need to be extrapolated from data developed for another purpose or limited data that are not representative) or altogether non-existent. Given that complete data and information are rarely available for microbial exposure assessment, you may need to make simplifying assumptions. Such assumptions result in uncertainty about exposure estimates. To support better risk management decisions and to provide transparency, the risk assessor characterizes uncertainty objectively.

This chapter provides general principles and practical guidance for conducting exposure assessments for microbial hazards. Information is organized into five sections: 1) general concepts and factors in exposure assessment, including discussion on variability, uncertainty, deterministic and stochastic risk assessment, and Monte Carlo analysis (section 5.1); 2) developing an exposure assessment (section 5.2); 3) analyzing results from a model (section 5.3); 4) communication, review, and validation of model results (section 5.4); and, 5) future developments in exposure assessment (section 5.5). The chapter also is intended to provide information useful to risk assessors, risk managers, decision-makers, risk communicators, stakeholders, general public, and researchers. Other resources that provide overviews of exposure assessment are those by Haas et al. (1999), the European Commission Scientific Steering Committee (ECSSC, 2003), Cox (2006), FAO/WHO (2008), Schaffner (2008), and Vose (2008).

5.1 What are General Concepts in Exposure Assessment?

5.1.1 What is an Exposure Assessment?

An exposure assessment is the process of estimating or measuring the magnitude, frequency, and duration of exposure to a microbial hazard(s), along with the number and characteristics of the person or population exposed. You can provide either a qualitative or quantitative evaluation of exposure; however, a quantitative evaluation is always preferable if the data exist and a quantitative risk assessment is needed. Ideally, an

exposure assessment describes the sources, pathways, routes, and the uncertainties about exposures.

Exposure comprises the sources, mode, route, and extent of contact the host has with the microbial hazard(s) of concern. Frequency of exposure describes how often a person is exposed. The duration is the length of time that a person is exposed to a microbial hazard.

MRA is typically concerned with characterizing the risk of single event exposures. But many environmental exposures are recurring events rather than single events. For example, a contaminated water source may be contaminated for days, or fomites may have infectious agents on them for days before they are cleaned or the agent dies off. Therefore, the microbial risk assessment may model repeated exposures, particularly if it includes secondary transmission. The dose refers to the number of microorganisms that correspond to a single exposure. The exposure dose constitutes the total number of organisms in a set of exposures. Simultaneous exposure to multiple hazards is also a concern in MRA.

5.1.2 What are Sources, Pathways, and Routes of Exposure?

There are various terms used to discuss the origin, movement or spread, and final intake of microorganisms by individuals or populations. Generally, the overall terminology refers to routes of transmission for microorganisms.

The source is the entity (or entities) that supply microorganisms to a particular exposure route. The source of microorganisms could be infected food animals, industrial processes, the environment (water, air, soil), or infected persons. The route of exposure (or route of intake) is the point where the microorganism comes into contact with the host. The three common routes of exposure are oral, inhalation, and dermal.

The physical movement of microorganisms, over time, from their source to the occurrence of an exposure is the exposure pathway or route of transmission. Exposure pathways may be complex; exposures may occur via aerosolization, water, food, soil, fecal-oral, and/or inanimate sources. The mode of transmission can be wind, flowing water, equipment movement, or vector organism. Not all modes of transmission are relevant for all exposure pathways. For example, neither exposure by inhalation via the nose or skin is highly relevant for foodborne exposures, but may be for water exposure. The number of microorganisms in a particular medium can increase or decrease across time as a function of changing environmental conditions, throughout the exposure pathway.

<u>Elements of Source Evaluation</u>

While sources of microorganisms may be living or inanimate, the elements of source evaluation are basically the same, with the caveat that not all modes of

transmission are relevant for all exposure pathways. A farm-to-fork model should consider:

a) How many viable pathogens (or indicators) are present in the source (e.g., infected chicken, contaminated carcass) at time zero?
b) How many pathogens are released from the source and/or what is the prevalence of infection in the source?
c) Over what period are the pathogens released?
    1) Continuously
    2) Batchwise
d) At what rate are the pathogens released?
    1) Counts/unit time (e.g., cfu, pfu, genomes per minutes, seconds, hours, days)
e) What is the form of the release?
    1) Fomites
    2) Spray equipment
    3) Offgases from a fermentor
    4) Waste water
    5) Animal slaughter
f) To what medium are they released?
    1) Food
    2) Surface water
    3) Soil
    4) Air
    5) Other surfaces

The evaluation of movement from a source into an exposure pathway is sometimes called a release assessment. The environmental release assessment identifies the sources of potential release, the media of release (air, water, or land), and the magnitude and frequency of release. The release estimates serve as inputs to the assessment of survival and distribution subsequent to release.

When there are data available to predict releases, four main steps are used in constructing the release assessment.

a) First, collect and synthesize information on how many organisms are generated at the site of release. For example, land application of biosolids would include the size of each load, the number of loads per unit time, and the concentration of organisms in a load.

b) Second, develop a process description to locate the places where releases may occur. For an industrial process, identify where and how the microorganisms are grown, and how they are separated from their growth medium. The process description also should consider the circumstances that would inactivate or destroy microorganisms. Inclusion of process flow diagrams can be useful for these purposes (e.g., as in a conceptual model).

c) Third, relate each possible point of release to the process involved. In agriculture, this could be a drop spreader or spray nozzle. In an industrial plant, it could be off-gassing (during separation, as from a centrifuge) from equipment during clean-up or during product transportation.

d) Fourth, develop quantitative estimates of release for each release source, which specify the amount of release, the time frame of release, and the media of release. If inactivation procedures or engineering controls are applied to the release source, then their effectiveness will need to be estimated to quantify the amount released after the control or treatment.

When intentional or incidental releases occur from inanimate sources, quantitative estimates can frequently be obtained. Releases to air from sources, such as a fermentor's off-gas, can be measured as the viable count per site per unit time. You can treat these as point source releases occurring at approximately rooftop height. A similar approach can be employed for wastewater releases. Modeling of release modes is usually medium specific; the output may be useful in estimating dispersal of the microorganisms from their source. Incidental releases may be modeled based on empiric evidence compiled for specific activities, but source evaluations of intentional releases to the environment are often complex and case specific.

The dynamic nature of microorganisms is one characteristic that differentiates microbial exposure assessment from chemical exposure assessment. Predicting changes in the number of microorganisms along an exposure pathway is often necessary to accurately estimate exposure doses. Environmental conditions that can influence the growth and decline in the number of microorganisms present in a specific media include, but are not limited to:

a) water activity,

b) pH,

c) carbon source for cellular constituents and energy,

d) electron acceptor,

e) sunlight intensity,

f) temperature,

g) population density of the microorganisms and/or other microbiota that compete for nutrients in the media or support pathogen growth, e.g., within biofilms on surfaces, and/or

h) presence of disinfectants or antimicrobials.

Depending on the characteristics of the microorganisms, some survive throughout an exposure pathway while others do not.

Many microorganisms have more than one exposure pathway and corresponding route of intake, often referred to as an exposure route or route of entry. Common exposure routes are:

a) inhalation (nose and mouth to lungs),

b) ingestion (oral intake of food, water, soil, and inanimate objects), and

c) direct (via skin, eyes, ears, inanimate objects, hand-to-hand, and sexual contact).

Some microbial exposure assessments are able to characterize exposure via a primary exposure pathway (e.g., *E. coli* O157:H7 from infected cattle to an individual via undercooked ground beef). Nevertheless, microbial and epidemiological evidence may indicate that some microorganisms spread via cross-contamination pathways; these pathways are sometimes difficult to characterize. Also, many microorganisms (e.g., noroviruses) are spread from an infected person to a non-infected person. This exposure pathway may be poorly characterized because data are often lacking (Zhao, 1998).

In addition to ingestion routes, chemical risk assessment is well developed for inhalation and dermal exposure routes. For example, EPA has many guidance documents for chemicals, such as *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry, Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment) Final* (RAGS-E), and the *Exposure Factors Handbook* (EPA, 1994, 2004c, 2011b). These references provide inhalation and direct (e.g., dermal) exposure routes and to the degree chemical guidance that might be useful for MRA; this information will not be discussed here.

5.1.3 How are Fate and Transport Considered in Exposure Assessment?

Fate and transport can be an important part of exposure assessment. Watersheds usually contain a mix of fecal and pathogen sources, much of which is not a concern until it is mobilized into waterways used for recreation or as a source for drinking water. Sporadic sampling of waterways, unassociated with periods of likely pathogen mobilization, provides a poor estimate of potential levels of pathogens of concern (Signor

and Ashbolt, 2006). Furthermore, it is well known that during rain events the predominance source(s) of mobilized fecal load may change, even during the river hydrograph. Given the uncertainties in the representativeness and value of infrequent grab sampling, modeling the fate and transport of pathogen/fecal indicators in source waters is considered a valid alternative to provide pathogen occurrence data for microbial risk assessments that examine different exposure scenarios.

Various modeling tools have been used or adapted to estimate watershed pathogen and fecal indicator fate and transport. The vast majority of models have focused on fecal indicators (Jamieson et al., 2004; Kay et al., 2010). Models that also include pathogens have typically been process-based. These models address a single pathogen (Walker and Stedinger, 1999) or multiple pathogens and fecal indicators (Dorner et al., 2006; Ferguson et al., 2007a) from fecal sources/loads on land (Ferguson et al., 2009), their mobilization during rain events (Ferguson et al., 2007b), and travel time to points of exposure so inactivation kinetics can be included. Environmental fate of pathogens is predominantly related to ambient temperature, biotic activity, and sunlight (e.g., Davies et al., 2005), each of which can be included as parameters in watershed models focusing on exposure of potentially infectious pathogens (Ferguson et al., 2010).

In situations when no or only very limited pathogen data are available at the point(s) of exposure, it may be appropriate to use pathogen surrogates to estimate pathogen (fate and transport) behavior in the environment. Surrogates may include particle removal during water filtration processes, such as to estimate *Cryptosporidium* removal, or concentration-time from accepted performance tables for a particular disinfectant. Microbial surrogates also have been applied, such as *E. coli* to represent bacterial pathogens, bacterial spores for *Cryptosporidium* oocysts, and bacteriophages for human enteric viruses (Medema et al., 2006). A related approach is to use the ratio of a surrogate to pathogen in a fecal source and assume the same range in ratio at the point of exposure, such as using enterococci to estimate the range of fecal load in a recreational water, and using that load range to estimate the range of possible pathogen occurrence (Schoen and Ashbolt, 2010).

5.1.4 What Environmental Factors Can I Take into Consideration?

Environmental factors are considered when the risk assessment is at the stage of calculating the amount of pathogen that constitutes an exposure. Examples of environmental factors are provided below with a short description.

a) **Ecological Niche** – An ecological niche is the abiotic and biotic elements in the environment that determine a species' chances of survival. Changes in the elements of an ecological niche determine whether an organism will increase or decrease in number.

b) **Gradients of Concentrations** – Microorganisms are rarely distributed equally throughout a medium, therefore gradations of concentrations usually need to be

considered in accordance to the media being assessed and the exposure being characterized.

c) **Persistence** – Persistence in the environment of an organism (i.e., spores) can be considered. Those organisms that are not stable in the environment would pose a different exposure, and subsequent risk, than those that are stable. (See Chapter 3)

d) **Matrix Characteristics** – The characteristics of the matrix where the pathogen is found may determine the amount and state (e.g., dormant, alive, dead) of microorganisms available to a receptor. Conditions such as oxygen content, fat content, pH, temperature, water content influence the survivability of microorganisms.

5.1.5 What is an Exposure Scenario?

An exposure scenario (or hazardous event) is the set of conditions or assumptions about sources, exposure pathways, amounts or concentrations of microorganisms, and the characteristics of the exposed individual, population, or population that constitute one or more exposures. An exposure assessment may be comprised of many different scenarios. Each scenario is the basis for evaluation and quantification of exposure(s) in a given situation.

A scenario analysis consists of a series of "what if" options for mitigation measures, interventions, or policy changes. This type of analysis allows for the evaluation of public or environmental health benefits of various measures that prevent or mitigate exposures.

5.1.6 What are Qualitative and Quantitative Exposure Assessments?

A qualitative exposure assessment is based on data and information which, when considered along with expert knowledge and identification of attendant uncertainties, provides a characterization of exposure in descriptive terms (e.g., high, medium, or low). A qualitative exposure assessment is necessary when there are not sufficient numerical data to develop a quantitative exposure assessment or if there is no acceptable method to translate human behavior or activities into quantitative terms.

A quantitative exposure assessment provides numerical expressions of exposure. Such an assessment provides numerical estimates of the likelihood of different microbial dose amounts, as well as numerical measures of confidence about its estimates (i.e., uncertainty).

Sometimes multiple quantitative exposure assessments are conducted for a microbial hazard in order to rank sources, vehicles, and/or pathways of exposure based on risk. One example of such an approach is a risk assessment of *L. monocytogenes* in ready-to-eat foods (FDA/USDA/CDC, 2003).

5.1.7 What is Variability in Exposure Assessment?

Variability describes a range of possible events. Variability in exposure can be caused by differences in location, activity, and/or behavior of exposed individuals at a particular point in time. These sources of variation result in differences in exposure to a microbial hazard(s) in various media. Variability is also caused by differences in the initial occurrence of microorganisms in various media (e.g., air, soil, food, and water). Because microorganisms grow and decline within media along the exposure pathway, there is variability in the amount of microbial hazard per unit of media intake by an individual or population subgroup. You may need to characterize variability in:

a) the number of microorganisms initially present in the medium;

b) the environmental conditions in which microorganisms exist;

c) the processes through which microorganisms move within scenarios;

d) the dose of microorganisms per unit of intake (e.g., serving of food, inhalation unit, amount of water ingested; spatial and temporal variability);

e) the amount of intake (inter-individual variability in exposure);

f) exposures across time (temporal variability); or

g) exposures across geographic location (spatial variability).

The types of variability considered depend on the type of exposure assessment to be developed as part of the overall risk assessment.

5.1.8 What is Uncertainty in Exposure Assessment?

Uncertainty is imperfect knowledge. You can reduce uncertainty by accumulating more information. Uncertainty may reflect imperfect knowledge of the microbial hazard (e.g., its virulence), environmental pathway/processes, or the human populations under consideration. Sources of uncertainty fit into two broad categories:

a) Uncertainty regarding one or more parameters in an exposure assessment (parameter uncertainty)

b) Uncertainty as a result of incomplete information or scientific theory needed to fully define the causal bases of exposures (structural model uncertainty)

Availability and quality of data and information can reduce the amount of uncertainty in exposure estimates. Objective depictions of uncertainty improve the transparency of information used by decision-makers in managing risk. The process of interpreting the influence of uncertainty on the results of an exposure assessment is referred to as uncertainty analysis.

5.1.9 What is a Deterministic Exposure Assessment?

You can conduct a quantitative exposure assessment using "most likely" or "conservative" values for the variables and uncertain parameters included in the set of scenarios. However, the use of single point estimates is not a preferred approach to inform decision making unless data are not available (EPA, 2002b; OMB, 2007b). These values are often referred to as point estimates and can result from collapsing the variability and/or uncertainty about random variables or parameters into singular values. Depending on how the point estimates are designed, the results may either represent an average or other extreme exposures (e.g., 95th percentile) among a specified population.

The use of point estimates in an exposure assessment is referred to as deterministic (static) modeling. Point estimates do not account for variability in the occurrence of the microorganisms at the source, variability in growth and/or decline in the number of microorganisms through the exposure pathway, or variability in intake across the population of individuals exposed to the microorganisms. Furthermore, a deterministic exposure assessment does not explicitly characterize the uncertainty about exposures. Without explicit characterization of variability and uncertainty, it is possible that point estimates will substantially over- or under-estimate exposures. If highly conservative point estimates – thought to be protective of public health – are used, the deterministic results may be characterized as worst-case estimates.

In some cases, deterministic modeling may be used to simplify the modeling of a highly complex system. For example, extensive modeling of transmission processes among a population may require simplifying assumptions about contact frequencies and transmission coefficients.

Another use of deterministic modeling is during your initial analysis of an exposure assessment model. Propagating simple numbers through compartments of the model or the full model may help with error-checking the mathematics of the model. Also, such calculations can provide early indications of the importance of various model components or pathways. Nevertheless, conclusions from such screening analyses should be cautiously interpreted because omission of variability can generate misleading results.

5.1.10 What is a Stochastic Exposure Assessment?

In contrast to using point estimates, the use of probability distributions for each parameter in an exposure assessment is preferred. A probability distribution includes both a range of values and the likelihood of occurrence for each numerical value. Use of probability distributions throughout the exposure assessment allows for the representation of variability in exposures of individuals and/or population subgroups. Probability distributions characterize the uncertainty in exposure assessments. When developing a stochastic model, use point estimates to verify the mathematical formula or confirm that

the computer code actually performs correctly for trivial cases (for example, the microbes are not present in the output if the initial concentration is zero).

Stochastic modeling refers to the use of probability distributions in an exposure assessment. Probability distributions represent the variability and uncertainty inherent in a system. Stochastic modeling can provide more realistic results by accurately characterizing the impacts of known sources of variability and uncertainty on risk estimates. Risk assessments are often concerned with the occurrence of rare events and stochastic modeling may reveal rare but consequential results (e.g., the occurrence of an unlikely, but large, population outbreak.)

Stochastic models often use computer simulations to mathematically combine multiple probability distributions in an exposure assessment calculation. Monte Carlo analysis (more detail in next section) is the most widely used probabilistic method to estimate these combinations. Advanced Monte Carlo modeling techniques also can quantitatively characterize uncertainty in exposure estimates (Gilks et al., 1996).

Stochastic modeling is usually more resource-intensive than deterministic analysis. Defining model inputs as probability distributions can require additional steps in the planning, review, and communication of the exposure estimates. It is important to maintain transparency when probability distributions are used to characterize model inputs. Use of probability distributions, however, provides a framework for incorporating more of the available information into an exposure assessment.

If data for critical variables and parameters are available, consider stochastic modeling for the exposure assessment if time permits. Alternatively, if data and information are insufficient, consideration should be given to the use of other modeling techniques. For example, the use of interval mathematics or fuzzy mathematics may provide a more credible assessment of the probabilistic boundaries of exposure than standard methods using probability distributions (Ferson, 1996).

5.1.11 What is Monte Carlo Analysis?

Monte Carlo analysis is a commonly used quantitative technique for exposure assessments. It involves the random sampling of each of the probability distributions in a model to estimate the likelihood of the model's possible results (Vose, 2008). Each re-calculation of the model is an iteration, and a set of iterations constitutes a simulation.

A cardinal rule of this analytical technique is that every iteration should be possible in nature (Vose, 2008). If followed, this rule can prevent errors in modeling logic. For example, a predicted serving cannot contain 2.7 microorganisms, although the average concentration across some volume or mass may be 2.7 microorganisms. While it is not possible that any individual would be exposed to exactly 2.7 (viable) organisms, the simulation can be acceptable if the exposure distribution is defined and sampled correctly. If a Poisson (discrete) distribution with a mean of 2.7 is used in the risk

modeling, the individual Monte Carlo iterations will all take discrete (integer) values, maintaining the realism of each iteration and of the simulation as a whole.

Three common problems are inherent in the Monte Carlo methods:

a) First, correlations and dependencies between variables may be unknown. If dependent variables are mistakenly assumed to be independent in a Monte Carlo analysis, the likelihood of common occurrences in the real world may not be correctly estimated via simulation (i.e., EPA, 1995). If information on the correlations is not available, then alternative methods (e.g., interval or fuzzy mathematics) may avoid such mistakes because the probability boundaries calculated by these methods can include a full range of correlations in their results (Ferson, 1996).

b) Second, the data necessary to estimate input distributions may be incomplete or entirely lacking. Although inadequate data are a problem for any exposure assessment method, Monte Carlo methods are particularly disadvantaged because these methods require explicit definition of the model inputs. Sometimes uniform or triangular distributions are used when data are sparse.

c) Third, the mathematical structure of the exposure assessment model may be questionable. Risk analysts often acknowledge the limitations induced by these problems and employ sensitivity analysis (or other methods) to assess their influence on estimated exposures (Law and Kelton, 2000; Vose, 2008). Care should be taken to make sure that uncertainties related to model specification are addressed by comparing the quality of fits across different model forms. Where sufficient data are available, methods such as cross-validation may be used. Using this approach, an exposure model is estimated using a portion of the data (usually about 70 percent) and then the model is tested for consistency with the remaining data set.

Examples of agency guidance on probabilistic risk assessment include EPA's *Guiding Principles for Monte Carlo Analysis* (EPA, 1997) and EPA's *Using Probabilistic Methods to Enhance the Role of Risk Analysis in Decision-Making With Case Study Examples* (EPA, 2009b).

5.1.12 How does Exposure Assessment Fit with the Other Components of Risk Assessment?

Fundamentally, risk assessment is a predictive analysis. It intends to "envision how the future will turn out if we undertake a course of action…" (Kaplan and Garrick, 1981).

Predictions are accomplished by answering three questions:

a) What could change?

b) How likely is that to happen?

c) What are the consequences given that it does happen?

The set of answers to the first question outlines the mutually exclusive scenarios to be considered in an exposure assessment. These scenarios (given the symbol s_i in the example developed here, where the index i implies there are potentially many) are mutually exclusive scenarios wherein something goes wrong ($i = 1$ *to* N). Each scenario that is identified has an associated probability that it occurs (l_i), as well as some measurement of its consequence (x_i).

Commonly, planning and scoping and hazard identification will determine the scenarios to consider for the exposure assessment (refer to Chapters 2 and 3). It is imperative that hazard identification establish the biologic plausibility of causative mechanisms considered in any exposure assessment.

Exposure assessment, therefore, is used to determine the likelihood of scenarios and a provisional consequence of the scenarios. In the context of exposure assessment, the provisional consequence is the magnitude of human exposure (usually dose) that occurs at the end of the scenario.

The output of the exposure assessment is commonly combined with the dose-response relationship developed in hazard characterization to predict the probability of an adverse human health outcome. This combination is considered in the risk characterization (Chapter 6).

In the assessment, order the scenario microbial doses from smallest to largest (i.e., $x_1 \leq x_2 \leq ... \leq x_N$) and similarly align the scenarios and likelihoods for each of those doses. This organization conveys the likelihood of increasingly larger microbial doses, as well as the scenarios that are responsible for those doses. Likelihood is a number constrained between zero and one. Because the $s_1, s_2, ... s_N$ are defined as mutually exclusive[27] scenarios, equation 5.1 follows:

$$\sum_{i=1}^{N} l_i \leq 1 \qquad \text{(Eq. 5.1)}$$

(if all s_i represent an exhaustive list of the things that can happen, then this sum will exactly equal one).

[27] Note that each scenario may include exposures through more than one pathway. Each scenario includes defined combinations of exposures that are sufficiently different to justify separate consideration.

In some exposure assessments, l_i will refer to the fraction of all possible exposures, including exposures with a dose of zero that derive from scenario s_i and generate a microbial dose of x_i. If the total exposures for the microbial hazard are known that can occur in one year (M), then the frequency of microbial dose x_i is $F(x_i) = M \times l_i$ (i.e., the number of exposures with a dose of x_i in one year is the product of total exposures and likelihood).

Given the frequency of microbial doses, the exposure assessment can directly generate a frequency distribution for human exposures (e.g., Figure 5.1). This distribution is simply a graphic display of the paired $\{x_i, F(x_i)\}$ values.

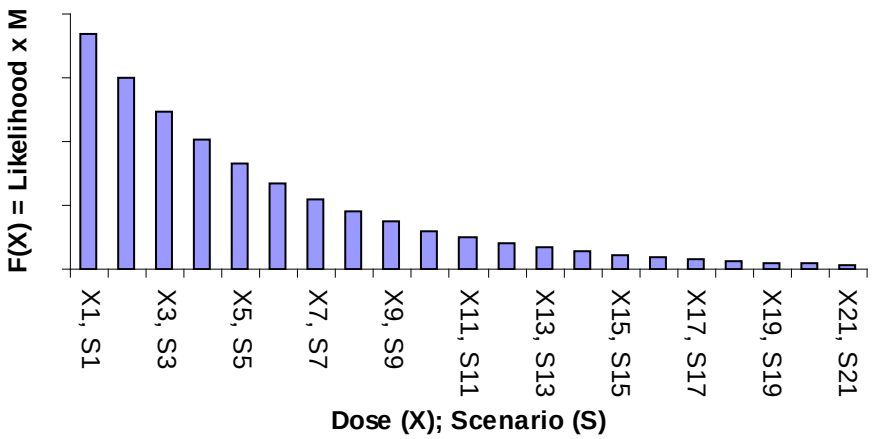

Figure 5.1 An illustrative exposure distribution developed by ordering scenarios (s_i) by the size of dose (x_i) and calculating frequency as the product of total exposures (M) and likelihood of the scenario (l_i).

5.1.13 Do Different Exposure Scenarios Always Generate Different Microbial Doses?

Although exposure scenarios may be mutually exclusive (i.e., represent unique pathways for microbial exposure), it is not necessary for different scenarios to result in different magnitudes of exposures. For example, consider consumption of water from a single source to which a treatment, with variable effectiveness, is applied. One scenario might reflect highly effective water treatment and consumption of a single 12-ounce serving that contains no microorganisms. Another scenario might reflect less effective water treatment and consumption of a serving of 6-ounces that contains no microorganisms. Although the scenarios are different, the resulting dose (0 microorganisms) is the same.

The process of developing an exposure assessment can be complicated; it is not always understood what scenarios will generate a given dose before the analysis begins. It is possible to design the exposure assessment such that scenarios are grouped by the

dose they generate. Alternatively, the scenarios can be described *a priori*, and the exposure doses they generate subsequently determined analytically. Depending on how scenarios are defined, it is possible that one exposure scenario may be associated with multiple doses (and their attendant likelihoods).

Consider a scenario where a healthy 30-year old male consumes a hamburger patty that was initially contaminated with 100 *E. coli* O157:H7 organisms and stored at 60˚F for 24 hours and then cooked at 130˚F (note: ground beef should be cooked to 160° F) for 4 minutes. The likelihood of this scenario might be 0.00089 percent of all servings of ground beef consumed by such a person. The consequence, from an exposure perspective, might be that 1,000 *E. coli* O157:H7 organisms are ingested. This scenario, when accompanied by all other scenarios for hamburger patties, is useful because it provides sufficient detail for decision-makers to appreciate the risk of adverse human health outcomes from consuming hamburger patties (especially once the risk characterization is completed). Ultimately, the purpose of risk assessment is to support decisions regarding risk and those decisions typically hinge on the three elements contained here:

a) What are the important scenarios?

b) What are the magnitudes of their consequences?

c) How likely are such scenarios to occur?

Short contemplation of this example, however, will undoubtedly raise many questions. How was the scenario identified; surely there are thousands of scenarios at that level of detail (e.g., what about a hamburger that is cooked at 129 degrees Fahrenheit [°F] for 2.5 minutes)? How was the likelihood determined? That likelihood seems very precise; do all servings of hamburger from this scenario have the same likelihood of occurrence? Why does an exposure of 1,000 microorganisms result from this scenario? Do all hamburgers handled this way have the same number of microorganisms at consumption? What are the important uncertainties in the estimate? Are there parts of the scenario that are more influential on the consequence than other parts?

The remainder of this chapter outlines general answers to these questions by discussing the process of developing, analyzing, and reporting an exposure assessment. These sections address scenario development, calculation of the likelihoods of scenarios, predictions of exposure doses, uncertainty analysis, and interpretation of exposure assessment results. Nevertheless, the ideas and concepts in these sections are not intended to be dogmatic prescriptions for completing an exposure assessment. In exposure assessment, there are many different approaches that may be valid for solving a problem. Yet, all valid approaches share some fundamental similarities. These similarities are the focus in the remainder of this chapter.

The concepts of scenario, likelihood, and consequence are fundamental to developing an exposure assessment. When analyzing an exposure assessment, the

concepts of variability and uncertainty are crucial. Finally, effective communication, transparency, and validation are essential considerations when reporting the findings of an exposure assessment.

5.2 How do I Develop an Exposure Assessment?

As discussed in Chapters 1 and 2, the beginning of the exposure assessment starts in planning and scoping. This important step lays the foundation for a successful exposure assessment.

In general, the exposure assessment should be as simple as possible while still including the important sources and steps leading to the exposure of concern. Based on "problem formulation" (see section 2.1.1), make decisions regarding the approach to exposure assessment (e.g., attribution modeling or process modeling, empirical modeling of epidemiological data, probabilistic or deterministic, dynamic or static) and structure of the assessment model (which pathways, single or multiple models) (Hurd and Kaneene, 1993). Clearly document what sources of data were considered, utilized, and omitted, and provide justification for those decisions.

Given the complexity of many exposure assessments, the process becomes a multi-disciplinary collaborative effort. Subject matter experts are regularly consulted and their judgment incorporated into the process. For example, the exposure scenario may include unique behaviors or activities that need to be described by someone who is familiar with the specifics of the exposure in question.

Conceiving an exposure assessment can be daunting. For this reason, some structure is needed. It is useful to begin by describing a conceptual model with all the necessary scenarios, followed by a full mathematical development of the conceptual model and, finally, by collection and analysis of data necessary to inform the model inputs identified in the mathematical model.[28] This structure theoretically ensures adequate attention to, and scrutiny of, the exposure assessment in an ordered and efficient manner.

Efficient exposure assessment is enhanced when precedents exist and are used. For example, beginning with already published conceptual models that require minor modification for a new application avoids unnecessary duplication of effort. Following reasonable precedents is also how standard methods can evolve.

5.2.1 What is the Purpose of the Exposure Assessment?

With respect to purpose, most risk assessments can be categorized into two broad categories – retrospective or prospective. A retrospective purpose applies to microbial hazards that are well-established as occurring sporadically or epidemically. A prospective purpose for a risk assessment applies to potential microbial hazards for which

[28] Note that this conceptual model may be software driven and is different than the conceptual model that is developed in planning and scoping which illustrates the broad overview of how risk happens.

the adverse human health effects are not established. This categorization scheme is important because a different series of questions should be considered prior to developing the exposure assessment depending on the purpose category (see Table 5.1). These questions are not exhaustive, but the answers will guide the development of an exposure assessment appropriate for informing specific risk management decisions. In addition, answering these questions may also identify information requirements and methods for collecting this information.

Table 5.1 General Questions Considered Prior to Conducting an Exposure Assessment

Prospective Purpose	Retrospective Purpose
Is disease onset only a potential at this point, or is there time to provide an answer?	Is disease onset imminent or already occurring, thus requiring an immediate answer?
Should the exposure assessment be structured as an in-depth analysis using less conservative assumptions?	Should the exposure assessment be structured more as a screening analysis, using default and/or more conservative assumptions?
Should the evaluation focus on both long- and short-term exposures?	Should the evaluation focus only on short-term exposures?
Should the analysis focus on both low- and high-level exposures?	Should the analysis focus only on high-level exposures?
Should attempts be made to measure (sample) or model exposures within the body?	Should concentrations be measured (sampled) or modeled in the media of concern?
Should the evaluation consider all potential exposure pathways for that particular microbial agent?	Should the evaluation focus only on those exposure pathways of imminent concern?
Should the analysis attempt to consider aggregate and/or cumulative exposures to multiple microbial agents?	Should the analysis focus only on the microbial agent causing the adverse health impact and only this exposure?

5.2.2 Which Scenarios Can I Consider?

Scenario development is the conceptual and creative part of exposure modeling. It melds considerations of purpose and scope with established or putative causal pathways. Although standardized frameworks for some microbial exposure assessments have been suggested (e.g., for food safety applications, see Nauta, 2002), few hard and fast rules exist that describe what scenarios should be considered in an exposure assessment. Therefore, this section provides some general considerations and a few examples.

Despite the lack of standard scenarios, those included in a specific exposure assessment should be clearly communicated and understood. Explicit diagrams (e.g., conceptual models) can be developed early in a project and these diagrams can include detailed descriptions of the inputs, parameters, flow, and relationships of these components in the exposure assessment. It is useful to establish meaningful symbols within the diagrams to represent these model components early in the project. Consistent

use of symbols will encourage clear communication among risk assessors and risk managers.

Detailed diagrams with consistent symbols can be discussed with risk managers early and often in an exposure assessment project. This approach encourages open and clear communication of the modeling approach. Exposure assessment is an iterative and collaborative process; clear descriptions of the model with constructive feedback from persons outside the project will facilitate improvements at the conceptual model stage.

Exposure assessments should be as simple as practical, but not simpler. Although hazard identification may suggest that the chain of causation for exposure is complex, the exposure assessment should only incorporate the complexity needed for the purposes of the risk assessment. For example, the exploration of unproven causal relationships is rarely useful in exposure assessments.

Conceptually, an exposure assessment begins by considering the occurrence of the target microorganism at some place and/or time (i.e., its source). An exposure assessment could plausibly begin by characterizing the distribution of doses (or concentration of microorganisms) at the time of exposure. Such a beginning would not include consideration of sources and processes that produced the exposures. Nevertheless, this approach would be satisfactory if the purpose of the risk assessment was investigative and risk managers strictly sought estimates of the potential adverse human health events that could occur. Of course, it would only work if data are available to estimate the distribution of doses just prior to exposure.

Often exposure assessments begin by considering the occurrence of microorganisms at a place and time that is somewhat distant and prior to the actual human exposures. Availability of microbial occurrence data is one justification for where and when to begin the conceptual model. For example, if the only microbial data available refers to its occurrence prior to the application of some treatment process, then the exposure assessment may begin at that place and time. The purpose of the risk assessment is another justification for where and when to begin the conceptual model. For example, if the purpose is to set a regulatory performance measure for some treatment process, then the exposure model will need to begin its considerations of microbial occurrence at some point prior to the treatment process.

Once the beginning of the exposure assessment is determined, use fate and transport modeling (including hydraulic models for waterways) or predictive microbiology to determine how microbial occurrence changes before a dose reaches an exposed human. Processes refer to events or phenomena that influence microbial occurrence between the beginning and end of the exposure assessment. As discussed later, generic processes include growth, attenuation, mixing and partitioning. The planning and scoping and hazard identification stages of the project include the processes that are included in an exposure assessment.

Many microbial exposure assessments involving food or water will include three general sequential stages: bulk processing, bulk transport, and consumption (Haas et al., 1999). Including these stages is often necessary to examine the influence of factors (or covariates) on microbial levels at the point of exposure. This inclusion is especially important when the purpose of the risk assessment is to predict changes in risk from one or more changes in these stages. For example, a risk assessment that examines a policy to require more controlled refrigeration of a food after it is produced – to limit growth of the target microbe – will include the bulk transport stage in the exposure assessment so that the effect of the policy relative to current conditions is measurable. In addition, consider seasonality in the exposure assessment. For example, the risk of foodborne *Campylobacter* is higher during the summer season in the United States than during the winter, because of food handling issues and growth of the bacteria in the warmer environment.

To complete scenario development, determine how each exposed population will come in contact with the microorganism of concern. The three well-recognized routes of initial exposure are inhalation, ingestion, and direct contact. These routes will influence the mathematical scale of the model. For example, inhalation exposures will likely depend on concentration measures of microorganisms (e.g., microorganisms per cubic meter) while ingestion exposures may depend on tracking actual counts of microorganisms to determine the average number of microorganisms in a serving.

Numerous examples could be used to illustrate different approaches to developing exposure scenarios. Three such examples follow:

a) To estimate the risk of viruses in water, an exposure assessment considered the volume of water consumed and the average concentration of viruses per liter of water supplying a large city (Haas et al., 1993). In this exposure assessment, no attention was given to mechanisms potentially responsible for the average virus concentration or to factors that might cause variability in virus concentration across time or water supplies.

   For this example, a scenario could represent one possible combination of concentration (e.g., viruses per milliliter) and water consumed (e.g., milliliters per person per day). Therefore, the set of scenarios would include all possible combinations. Alternatively, this analysis is an example as a single scenario (e.g., water consumed) with variable average dose of virus per day.

b) To estimate the risk of tuberculosis (TB) transmission on a commercial airliner, an exposure assessment examined the spatial variability in concentration of this mycobacterium (Ko et al., 2004). This assessment included air transfer rates between different cabins in the plane, respiration rates of potentially exposed individuals, distance from an infectious source, and the rate of expired TB organisms from an infectious source. Hazard identification had indicated that all these factors might influence exposures of passengers to TB on a plane. The exposure assessment focused on the cumulative exposure to infectious TB during

a long (> 8 hours) intercontinental flight. For this example, scenarios reflected the combinations of cabin location of exposed individuals, location of source individual, and airflow direction.

c) To estimate the risk of human illness from *E. coli* O157:H7 in ground beef, an exposure assessment included causative factors during on-farm production, slaughter, and storage/preparation stages (Ebel et al., 2003). This complex farm-to-table exposure assessment examined the influence of season, live animal prevalence, transport, dehiding, carcass decontamination, carcass chilling, carcass fabrication, grinding, storage/handling, cooking, and consumption on the predicted exposure per ground beef serving. Hazard identification indicated these factors might influence exposures. Control of many of these factors was considered by risk managers.

   For this example, scenarios reflected the combinations of different sources (e.g., prevalence of different classes of infected cattle presented for slaughter by season) with effectiveness of decontamination procedures with times/temperatures of storage and cooking. The unique combinations that represent individual scenarios were too numerous to count. Furthermore, ground beef servings were created from combinations of scenarios that produced the beef that went into a load of ground product. Therefore, the exposure assessment did not list distinct scenarios but, instead, produced frequency distributions for levels of *E. coli* O157:H7 per serving of ground beef for low- and high-prevalence seasons of the year.

An exposure assessment depends on the microbial agent's properties and the environmental transmission factors relevant to exposures. Microbial agents may stem from more than one source, may be transmitted via multiple routes of exposure, and may be spread via secondary or vector transmission. An exposure source can originate from either natural or anthropogenic events, activities, or locations that generate or release hazards. Exposure sources can be classified as point sources or non-point sources. Exposure routes include inhalation (nose and mouth to lungs), ingestion (oral), and direct contact (skin, eyes, ears, and sexual). Exposure routes are situation-dependent and medium specific. An exposure pathway encompasses both exposure source and route, and generally is described by a source and release from a source, an exposure point, and an exposure route. Table 5.2 illustrates the exposure assessment components and their relationship to various exposure points.

Table 5.2 Transmission Pathways for Microbial Hazards

Source	Release	Exposure Point (vehicle)		Exposure Route[a]
Natural or Anthropogenic Point or Non-point	Natural Accidental Intentional	Food	• Meat • Poultry • Eggs and Egg Products • Fish and shellfish • Produce • Dairy Products • Other Food Products	• Ingestion • Direct Contact
		Water	• Surface Water (Drinking) • Ground Water (Drinking)	• Ingestion • Direct Contact • Inhalation
			• Recreational Water • Compost Tea[29]	• Incidental Ingestion • Direct Contact • Inhalation
		Soil	• Surface Soil • Subsurface Soil • Sediment / sand • Manure • Biosolids	
		Air	• Ambient Air • Indoor Air	
		Surfaces	• Porous • Non-porous	
		Biota	• Plants • Animals, including humans	

[a] Exposure routes include inhalation (nose and mouth to lungs), ingestion (oral), and direct contact (skin, eyes, ears, and sexual)

The dynamic nature of host-pathogen interactions, unique to infectious disease risk assessment, can lead to secondary transmission. The strictest definition of secondary transmission pertains to direct human-to-human contact between a primary case (infected or ill) and a secondary case that becomes infected or ill from that contact. Broader definitions include secondary cases that arise from contact with fomites, food, or water contaminated by primary cases. Where secondary transmission includes infection from

[29] Compost tea is made by soaking or steeping compost in water. The resulting compost tea is used for fertilizing plants.

pathogens in the environment (e.g., fomites), it would not be considered secondary unless it occurs in the context of an outbreak where primary cases have already been identified. In such a case, the term "secondary transmission" is not used in the strictest sense, but is commonly used by public health professionals in the context of an outbreak.

5.2.3 What are the Exposed Populations I Could Consider?

Identifying the exposed individuals or populations of interest is crucial to determining what data are needed for an exposure assessment. In some cases there may be statutory requirements or agency policies that require certain populations to be considered. The following factors are inherently tied to the exposure scenario:

a) **Exposure Duration and Frequency** – Certain individuals/populations may have a comparatively greater exposure duration or frequency in a given environment. For some microorganisms, it is possible that exposure to a low concentration for a long duration may be a concern even if the concentration would not pose any health risks under short-term exposures.

b) **Exposure Routes** – Knowing the characteristics of the exposed population helps develop the appropriate exposure pathways to consider in the exposure assessment. In addition, it can be helpful to think of exposure activities that may put someone at risk, such as fertilizing a garden with manure, cleaning out a cat litter box, visiting a petting zoo, foreign travel, living with a small child who attends daycare, and so on. Most of this type of information can be collected through questionnaires or interviews.

c) **Sensitive Individuals/Populations and Life Stages** – Some individuals/populations may be more susceptible to infection or more likely to develop severe manifestations of infection. For example, while healthy individuals may recover from an *E. coli* infection, it can be deadly to young children, the elderly, and people with compromised immune systems. Similarly, chronic smokers may have impaired mucociliary clearance mechanisms and, therefore, may be more susceptible to respiratory infections.

Once the exposed individual/population or life stage has been determined, it is important to include any scenario-specific conditions in the conceptual model. Sensitive populations and/or life stages (refer to Chapters 3 and 4) which may have different exposure considerations may include but are not limited to:

a) Young children (up to 10 different age groups [EPA, 2005a]);

b) The elderly;

c) Persons with compromised immune systems;

d) Pregnant women;

e) Chronic smokers;

f) Military personnel (deployed and non-deployed);

g) Occupationally exposed individuals;

h) Other groups based on behavioral patterns (e.g., individuals who subsistence fish or those who live in institutional settings with shared meals and bathrooms).

You should select individuals/populations of interest based on the purpose of the risk assessment. However, your selection may be limited by data availability. Any differences between the populations considered in one part of the assessment (e.g., the dose response) and another (e.g., the exposure assessment) component of risk assessment should be carefully explained and the implications of those differences should be discussed.

5.2.4 What Approaches to Exposure Modeling Can I Use?

Although there are more elaborate classification schemes (Hurd and Kaneene, 1993), most exposure assessments use an attribution and/or process modeling approach (Cox, 2006). Process modeling generates traditional results (i.e., likelihood and dose) from the exposure assessment. Attribution modeling, in contrast, does not explicitly estimate the likelihood of microbial doses. Instead, this approach implicitly synthesizes the exposure output with hazard characterization to generate links between model inputs and numbers of human illnesses. Some refer to attribution and/or empirically-based models as 'black-box' approaches because causative mechanisms are not explicitly included (ECSSC, 2003). Nevertheless, attribution modeling is empirically based and is often available to assessors when the problem is rich with surveillance evidence.

<u>Attribution Modeling</u>

Empirical estimates of the number of human illnesses per year caused by each microorganism are sometimes available (e.g., diseases covered by surveillance systems, reportable diseases, for example, for foodborne pathogens, see Scallan et al. [2011a,b]). These data might be used, in conjunction with other information, to back-calculate the fraction of illnesses attributed to various scenarios (Pires and Hald, 2010; Painter et al., 2009). Although epidemiological surveillance data are prone to data gaps that may not be well characterized (under reporting of illnesses is significant and varies), national surveillance systems combined with thorough investigations of large-scale epidemics may generate sufficiently valid conclusions about sources and causes of those illnesses. If such data are available, it is feasible to determine the number of exposures from a target source that cause human illnesses. Furthermore, the probability of illness per exposure can be estimated by dividing the target source by the estimated number of total exposures (e.g., total servings of a particular food commodity consumed per year).

Ultimately, the number of illnesses from a microorganism that are attributed to a source represents the total number of illnesses that could be avoided by eliminating that source. If complete elimination of the source is not feasible, then further analysis might suggest what fraction of a baseline number of cases could be avoided by a proposed risk management option that improves the effectiveness of some particular process.

For illustrative purposes, assume 1,000 cases of illness caused by microorganism B are detected by public health surveillance each year (Figure 5.2). Analysis of the surveillance system suggests that only one of every 50 cases that occur is detected via this system. Therefore, the true number of cases is estimated to be 50,000 per year. Epidemiologic evidence suggests that Source 1 (e.g., ground beef) causes 20 percent of cases. Research evidence suggests that 50 percent of Source 1 exposures are directly attributable to process 1 (e.g., under-cooking). Based on these values, you estimate the number of illnesses attributed to Source 1 is 50,000×0.20=10,000. You estimate the number of those illnesses attributed to process 1 is 10,000×0.50=5,000.

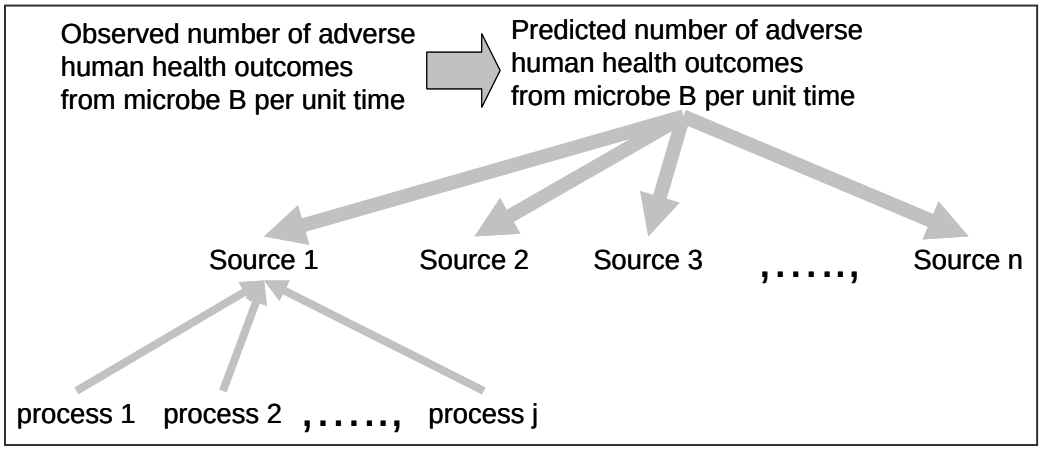

Figure 5.2. Schematic illustrating direction of inference when using an attribution approach to exposure assessment.

An attribution approach has been applied to food safety risk assessments (Bartholomew et al., 2005; USDA, 2008b; Withee et al., 2009; USDA, 2011; Williams and Ebel, 2012). This approach begins with illnesses reported to a surveillance system. More than 75 countries have implemented surveillance systems to monitor occurrences of foodborne illnesses (Herikstad et al., 2002; Allos et al., 2004; de Jong, 2006). In the United States, the FoodNet system serves this function (CDC, 2011). Nevertheless, these surveillance systems do not capture every case of foodborne illness, so under-diagnosis factors are developed to estimate the total number of illnesses for the pathogen of interest (Scallan et al., 2011a; Ebel et al., 2012). Additional scaling factors can be developed to extend these estimates to specific product-pathogen pairs (Hald et al., 2004; Guo et al., 2011; Williams and Ebel, 2012).

These attribution methods support estimation of the number of human illnesses occurring in a population of consumers during some period. Nevertheless, regulatory

agencies usually conduct microbial risk assessments to evaluate the change in human illnesses that results from a proposed intervention that intends to reduce the level of contamination in the food supply.

Williams et al. (2011a) explain a framework for quantitative risk models that is amenable to an attribution-based, Bayesian analysis (see section 4.2.1). The Bayesian question assessed in these applications is: what is the likelihood of a particular model form – or set of model parameters – given the available evidence regarding the observed occurrences of human illnesses associated with a particular food product?

Two basic model approaches – prevalence- and dose-based – are developed within this framework. Advantages of the framework are that estimates of human illnesses are consistent with national disease surveillance data (that are usually summarized on an annual basis) and some of the modeling steps that occur between production and consumption can be collapsed or eliminated. Use of the framework leads to probabilistic models that include uncertainty and variability in critical input parameters.

A Bayesian approach is usually computationally intensive, so simpler probabilistic models may be preferred. The proposed modeling framework is based on three primary determinants of adverse human health outcomes from foodborne pathogens; 1) the frequency of exposure to the pathogen; 2) the distribution of pathogens in a random exposure event on a per serving basis; and 3) the probability that a random exposure event causes the adverse human health outcome (Cox, 2006; Haas, 1996). In microbial food safety, sporadic exposure events are considered independent events and chronic exposures to pathogens are historically not considered (primarily due to lack of data). These characteristics support modeling the occurrence of human illnesses as a Poisson process, an assumption that simplifies the application of Bayesian solution techniques.

A prevalence-based model estimates changes in annual illness counts based on changes in the frequency of occurrence among food commodities (Williams et al., 2011a). Changes in frequency may be evidence-based (e.g., observed time-series data from surveillance of carcasses across a slaughter industry) or based on assumed changes from a proposed intervention.

The basic model is:

$$P(ill) = P(ill \mid exp)P(exp) \qquad \text{(Eq. 5.2)}$$

where $P(ill)$ is the probability of illness from a product-pathogen pairing across a population, $P(ill \mid exp)$ is the probability that exposure to a random contaminated serving

will produce illness[30], and $P(exp)$ is the frequency of exposure to the pathogen on a per serving basis[31]. Note that attribution evidence can inform $P(ill)$. If data also exist to estimate $P(exp)$, then Bayesian methods will estimate $P(ill \mid exp)$.

This basic model enables a simple estimation of annual illnesses avoided ($I_{Avoided}$) resulting from an intervention that reduces prevalence:

$$I_{Avoided} \sim Poisson\left[\left(1-\frac{P_{new}(\exp)}{P_{initial}(\exp)}\right)\lambda_{ill}\right] \qquad \text{(Eq. 5.3)}$$

where $I_{Avoided}$ depends on the ratio of new to old (initial) prevalence and the expected annual rate of illnesses (λ_{ill}) prior to the intervention estimated from attribution evidence.

The advantage of this modeling approach is that it obviates the need to estimate an exposure distribution or a dose-response relationship. Because estimating an exposure distribution is resource intensive – and data for dose-response relationships are notoriously limited – the use of a prevalence-dependent approach allows the analyst to focus attention on highly relevant evidence concerning changes in the pathogen's occurrence at the stage affected by the proposed intervention.

The critical assumption needed to apply a prevalence-based approach is that dose levels at consumption are independent of the frequency of contamination[32]. For example, if the within-herd prevalence of a pathogen is stable but the pathogen is clustered among herds, then changing the number of affected herds is expected to proportionally alter risk to consumers. This is because the factors that influence dose levels on servings, which occur after animals leave the herd, are not altered by the existence of more or fewer affected herds.

Although the prevalence-based model is not appropriate when there is clearly an association between prevalence of contamination and the levels of contamination (e.g., when the apparent prevalence of contaminated carcasses increases, so do the average number of pathogens on the carcasses), empiric evidence supports the independence of prevalence and contamination levels for some product-pathogen pairs. For example, in rinse samples of chicken carcasses that test positive, the average concentration of

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[30] $P(ill \mid exp)$ is the solution to the integral $\int_{>0}^{\infty} R(D)\, f(D)\, dD$ where $R(D)$ is the dose-response function and the exposure distribution of doses (D > 0 organisms) is the probability density $f(D)$.

[31] Exposure to a contaminated serving can be defined at any point in the farm-to-table continuum assuming that $P(exp)$ is proportional to the percent of positive units observed at some point prior to consumption (i.e., these measures of occurrence differ by a multiplicative constant). In food safety applications, the best data for measuring frequency is usually at the point of commercial production (e.g., retail-ready raw chicken carcasses).

[32] This assumption asserts that $P(ill \mid exp)$ is constant regardless of changes in $P(exp)$.

Salmonella per ml of sample rinsate was 0.16 and 0.14 cfu in the 1995 and 2007 baseline surveys, respectively (USDA, 1996; USDA, 2009). Yet, the prevalence of positive carcasses was demonstrably different (20% vs. 7.5%) in those surveys.

The independence of prevalence from consumed dose is evident in a risk assessment of *C. perfringens* in cooked meats (Crouch et al., 2009). In this case, high doses at consumption levels that are necessary for illness to occur are essentially independent of the initial pathogen levels prior to cooking. The high doses represent extreme events that result from inadequate refrigeration of meats post-cooking. For a random serving to be a high risk, it must be contaminated and mishandled. The actual level of pathogens among the contaminated servings prior to mishandling is essentially irrelevant because the substantial amplification of organisms that follows inadequate refrigeration swamps the initial levels.

Similar circumstances apply to other foodborne pathogens. For example, servings of ground beef – if contaminated – have generally low levels of *E. coli* O157 at the time of production (USDA, 2001). Yet, the risk at consumption is driven primarily by rare events that allow growth of the pathogen and rare occasions of substantial under-cooking. Furthermore, both of these rare events are independent of one another. Therefore, the risk of illness depends on a serving being contaminated and the probability that a contaminated serving was mishandled prior to consumption. If an intervention intends to modify the prevalence of contaminated raw meat products, the prevalence-based approach is often useful (Text Box 5.1).

Text Box 5.1. Example of a prevalence-based approach

Withee et al. (2009) assess the public health effects of a cattle vaccine intervention for *E. coli* O157. They derive relationships between prevalence of infected cattle and human illnesses by asserting that no human illnesses from beef products would occur if *E. coli* O157 did not occur amongst live cattle (i.e., prevalence = 0.0%) and assuming current human illness estimates are proportional to current live cattle prevalence estimates. A simple linear relationship between these two points (on a human illness vs. cattle prevalence chart) suggests that the prevalence reduction caused by applying a cattle vaccine will generate a proportional reduction in human illnesses. Using this relationship, the analysis considers the break-even costs of vaccine (for various combinations of vaccine performance) to inform decision-makers about the appropriateness of a vaccine intervention for this public health issue.

If the assumption underlying a prevalence-based approach is not reasonable, a dose-dependent approach can still yield simplified solution algorithms using attribution modeling. If exposure doses are generally small enough such that the dose-response relationship is essentially linear, and we further assume prevalence is unchanged by an intervention, then exposure modeling only needs to estimate the change in average dose to estimate the change in human health risk. That is, the illnesses avoided per year by an intervention that intends to reduce contamination levels can be modeled as:

$$I_{Avoided} \sim Poisson\left[\left(1-\frac{\bar{D}_{new}}{\bar{D}_{initial}}\right)\lambda_{ill}\right] \qquad \text{(Eq. 5.4)}$$

where $I_{Avoided}$ depends on the ratio of new to old (initial) average exposure dose levels and the expected annual rate of illnesses (λ_{ill}) prior to the intervention estimated from attribution evidence.

This dose-dependent simplification asserts that changes in illnesses are proportional to changes in average dose and obviates the need to model the changes for an entire exposure distribution (Text Box 5.2). Nevertheless, applying this equation hinges on the assumption that exposure doses occur within the linear portion of a dose-response curve. Williams et al. (2011b) address this issue for a number of common dose-response functions. That publication provides the methods for calculating the maximum dose level for which the linear approximation to the dose-response function is appropriate.

Text Box 5.2. Dose-dependent simplification

A dose-dependent simplification was used in a risk assessment of *V. parahaemolyticus* contaminated clams in Thailand retail establishments (FAO/WHO, 2002). In this application, fitting of available data to a beta-Poisson dose-response function suggested that this function was linear up to a dose of 6 $\log_{10}$. Because 99% of exposure doses were below this level of contamination, a simple dose-dependent assumption was made to estimate the change in risk that would result from interventions such as improved chilling of clams.

The assumption of low doses is not always tenable. Neverthless, the dose-dependent simplification may still be useful for estimating the risk associated with exposures in the low dose region. For doses exceeding the upper bound of the linear dose-response relationship, an algorithm that solves $\int_{>D_{\max}}^{\infty} R(D)\,f(D)dD$ is provided (Williams et al., 2011c). This algorithm is compatible with software packages commonly used in Bayesian estimation. It involves partitioning the dose-response function into discrete linear intervals, then using conditional expected values of the exposure distribution to approximate the integral. This approach provides more stable risk characterization estimates with less computation time than Monte Carlo integration approximations.

Attribution methods are only as meaningful as the evidence available to estimate attribution of human illnesses from a pathogen to a source. Although attribution methods are improving for the common foodborne pathogens (Hald et al., 2004; Pires et al., 2010; Guo et al., 2011), there remain microbial pathogens and routes of exposure for which data to estimate attribution are not available. In these cases, process modeling may be necessary, although thoughtful assumptions about attribution and other model inputs may

still provide rapid assessments of the bounds of risk when such estimates are needed for immediate decision-making (Cox, 2006).

5.2.5 How is Scenario Analysis Used in Exposure Assessment?

Scenario analysis begins the process of identifying those scenarios that constitute a risk as well as those that are not a risk. It simplifies the next stage of exposure model development, quantifying the likelihood of those scenarios that end in an exposure dose.

In developing scenarios during the conceptual development phase, begin by defining the mathematical relationships between process steps. Are these additive or multiplicative? Are there correlations between different inputs? What other factors influence the relationships among inputs?

Compared to scenario development, determining likelihood and dose is usually a more mathematical exercise. The relationships between model inputs need to be mathematically described; often statistical methods are employed to quantify these relationships. Also, the process of converting conceptual relationships into explicit mathematical relationships commonly involves additional assumptions beyond those represented in the conceptual model. Transparency is particularly important during this process.

Before data availability or analysis is considered, the mathematical development needed to determine likelihood and dose should be determined. Once a tentative mathematical model is completed, its structure can be communicated with outside reviewers and risk managers. Typically, the mathematics will provoke discussion about the data needs of the model. A clearly defined mathematical model provides an opportunity for specialists to review and comment on the course of the project. If scrutiny of the mathematics determines the need for change, then make changes before a large investment in data acquisition and analysis is completed. Frequent outreach and feedback is one manifestation of effective exposure assessment.

<u>An Illustrative Example</u>

In a very simple illustrative example, you want to predict the exposure risk from servings containing 10 units of microbe B (Figure 5.3). Two scenarios are considered: one where the serving is cooked such that all the microorganisms in the serving are destroyed and another where the serving is not cooked and all the microorganisms survive. From available data or expert opinion, you determine that the likelihood of cooking such servings is f, where $0 \leq f \leq 1$. Therefore, the likelihood (l_1) of the cooking scenario (s_1) is f and the likelihood (l_2) of the non-cooking scenario is $1-f$.

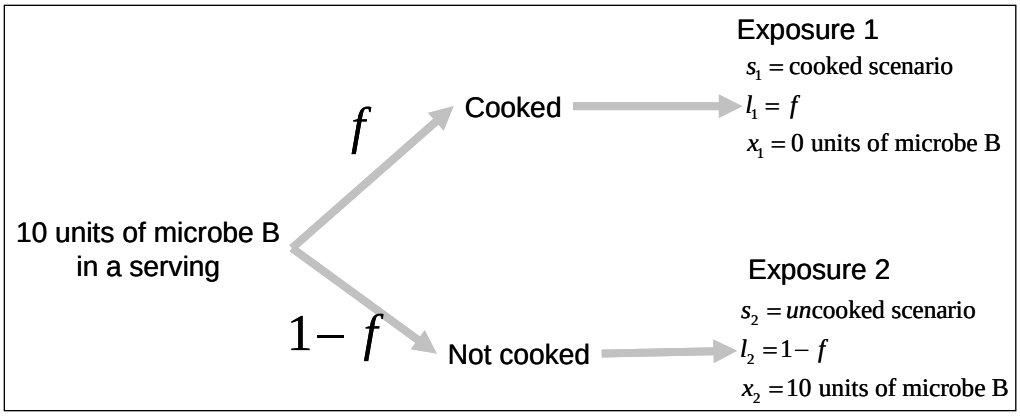

Figure 5.3 A simple illustrative example of two exposure scenarios resulting from an initial amount of microorganism B in a serving of food. In this case *f* is the probability that a serving will be cooked such that all of microbe B is destroyed. Conversely, 1-*f* is the probability that a serving will not be cooked and the total amount of microorganism B survives to expose a consumer.

In a slightly more complicated illustrative example, you consider three representative concentrations of microorganism B per serving, three levels of cooking effectiveness, and three amounts of food or water consumed per serving (Figure 5.4). This example generates 27 different scenarios for the exposure assessment.

You may assume this example pertains to some bulk product in which three average concentrations (e.g., microorganisms per milliliter or per gram) are possible. You define each concentration as λ_1, λ_2, and λ_3 and refer to the likelihood of each of these as $f(\lambda_1)$, $f(\lambda_2)$, and $f(\lambda_3)$. A similar approach is used for cooking effectiveness levels (ε_i and $g(\varepsilon_i)$) and consumption amounts (υ_i and $h(\upsilon_i)$) to define their values and likelihoods[33].

[33] Note that branch likelihoods must sum to one (i.e., $\sum_{1}^{3} g(\varepsilon_i) = 1$) so if we know two of the likelihoods then the third is also known (e.g., $g(\varepsilon_3) = 1 - g(\varepsilon_1) - g(\varepsilon_2)$.

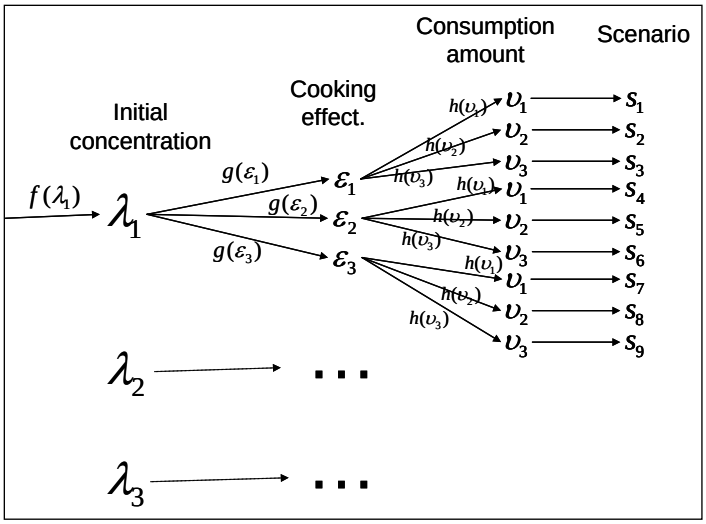

Figure 5.4 An illustrative example of a slightly more complicated exposure assessment

The dose and likelihood for each of the 27 scenarios generated for this exposure assessment can be calculated directly from the information given. For example, the average dose for scenario 1 (x_1) is the product of concentration (λ_1), cooking effectiveness (ε_1), and serving size consumed (υ_1). Similarly, the likelihood of scenario 1 (l_1) is the joint probability of each of these events occurring; if you know these events are independent, then $l_1\ (x_1,\lambda_1,\varepsilon_1,\upsilon_1) = \mathrm{f}\ (\lambda_1) \times \mathrm{g}(\varepsilon_1) \times \mathrm{h}(\upsilon_1)$.

Besides the complication of more scenarios, this example also uses a concentration measure for microorganism levels instead of a fixed number of microorganisms in a serving. Concentrations are used in many food and water microbial exposure assessments. It is sometimes recommended that modeling microbial concentration be avoided and physical counts of microorganisms should instead be explicitly tracked (ECSSC, 2003; Nauta, 2005). One justification for such an approach is that it avoids mass balance mistakes that can occur when processes necessarily change the microbial numbers. For example, a partitioning of some bulk quantity into subunits necessarily must account for all the organisms that existed in the bulk quantity. Yet, an average concentration per subunit might result in an incorrect assessment of the exposure dose per subunit. Nevertheless, for problems without partitioning or mixing processes, using concentration may be sufficient.

The multiplications for this simple model are common for many process models. Exposure dose is often the result of multiplicative, input-output, relationships; in this case the average dose for a scenario is $x_i = \lambda_i \times \varepsilon_i \times \upsilon_i$. The unit of x_i is microorganisms per serving; λ_i is microorganisms per volume or mass; ε_i is unitless ratios; and υ_i is volume or mass per serving. Predictive microbiology sometimes prefers to represent microbe quantities in $\log_{10}$ units. A logarithmic treatment will convert the multiplicative calculations of the model to addition or subtraction (e.g.,

$\log(x_i) = \log(\lambda_i) + \log(\varepsilon_i) + \log(\upsilon_i)$) while the joint likelihood remains the product of the likelihoods of each variable in the model.

Although the results from this example are trivial, it is illustrative to interpret them. These results explain which scenarios generate the highest doses and the likelihoods that those doses occur. By summing the likelihoods of scenarios in which zero organisms occur, the frequency of non-zero exposures can be determined.

The relative importance of different concentrations, cooking effectiveness levels, and amounts consumed also might be assessed by calculating conditional expected dose values. For example, the conditional expected dose value for one concentration ($E\left[x \mid \lambda = y\right]$) is the average dose calculated when only that concentration is considered but all the values for cooking effectiveness and amounts consumed are still possible:

$$E\left[x \mid \lambda = y\right] = \frac{y \times \sum_i \sum_j \left(\left(\varepsilon_i \times \upsilon_j\right) \times \left(g(\varepsilon_i) \times h(\upsilon_i)\right)\right)}{f(\lambda = y)} \qquad \text{(Eq 5.5)}$$

The magnitudes of the conditional expected values suggest the influence of these inputs on the average exposure dose (Table 5.3). For example, the largest change in average dose occurs across the possible values for initial concentration, but the smallest average dose occurs if cooking is completely effective.

Table 5.3 Results of a simple exposure assessment

λ_i	$f(\lambda_i)$	ε_i	$g(\varepsilon_i)$	υ_i	$h(\upsilon_i)$
1	0.7	0	0.75	10	0.25
10	0.2	0.5	0.2	100	0.5
50	0.1	1	0.05	150	0.25

Variable	Value	$E[x \mid variable = y]$
λ_i	1	14
	10	135
	50	675
ε_i	0	0
	0.5	249
	1	462
υ_i	10	12
	100	116
	150	173

	Calculated values		Sorted values	
scenario	dose,	likelihood,	dose,	likelihood,
s_i	x_i	l_i	x_i	l_i
1	0	0.131	0	0.750
2	0	0.263	5	0.035
3	0	0.131	10	0.009
4	5	0.035	50	0.080
5	50	0.070	75	0.035
6	75	0.035	100	0.020
7	10	0.009	150	0.009
8	100	0.018	250	0.005
9	150	0.009	500	0.021
10	0	0.038	750	0.010
11	0	0.075	1000	0.005
12	0	0.038	1500	0.003
13	50	0.010	2500	0.010
14	500	0.020	3750	0.005
15	750	0.010	5000	0.003
16	100	0.003	7500	0.001
17	1000	0.005		
18	1500	0.003		
19	0	0.019		
20	0	0.038		
21	0	0.019		
22	250	0.005		
23	2500	0.010		
24	3750	0.005		
25	500	0.001		
26	5000	0.003		
27	7500	0.001		

As the previous example illustrates, explicit tree-diagram schematics of exposure scenarios are daunting because the inputs can assume multiple values and the number of processes increases. A full graphic depiction of all 27 scenarios for the previous example would not fit on a single page.

For more complicated exposure models, the inputs are simply treated as random variables that are mathematically combined. In the simple example, represent all three possible values for initial concentration as $\tilde{\lambda}$ where the tilde symbol signifies that concentration is a random variable. You can similarly define $\tilde{\varepsilon}$ and $\tilde{\upsilon}$.

Using symbols for the random variables in the model, exposure can be mathematically written as $\tilde{x} = \tilde{\lambda} \times \tilde{\varepsilon} \times \tilde{\upsilon}$ where the dose delivered to humans is also a random variable by virtue of the fact that it is a function of random variables. Statistical moments of $\tilde{x}$ (e.g., its mean and variance) might be predictable, but when the likelihood distributions for $\tilde{\lambda}$, $\tilde{\varepsilon}$ and $\tilde{\upsilon}$ involve more than a trivial number of values, other techniques (e.g., Monte Carlo simulation) are often used to determine $\tilde{x}$. Nevertheless, the techniques essentially mimic the procedure followed for the simple example; the possible values for $\tilde{\lambda}$, $\tilde{\varepsilon}$, and $\tilde{\upsilon}$ are multiplied together and their joint likelihood is determined.

Once an exposure model becomes more complex, the identities of individual scenarios are more difficult to determine. It is common for exposure assessments to focus on predicting the likelihood of doses without explicitly identifying scenarios. In these situations, sensitivity analysis is used to sort out the relative influence of model inputs on the exposure distribution. Nevertheless, it is sometimes crucial to identify the higher risk scenarios; thinking about the model as a scenario tree is one useful technique for elucidating those scenarios.

Random variables can be discrete or continuous. Exposure assessments often use a mixture of both. Although it is intuitively appealing to consider microbial counts as discretely distributed random variables, it is not always essential that they be treated as such. Naturally continuous distributions, like weight measures or measures of effectiveness, may also be treated as discrete random variables in some models to simplify their calculations without any loss of information. Like most decisions in exposure assessment, planning and scoping inform the choice of distribution. Nevertheless, such decisions should be made with an understanding of the biologic plausibility of the choice.

Determining the likelihood of doses of microorganisms is the fundamental objective of most exposure assessments. The key to effective exposure assessment, therefore, is to explain the mathematical relationships among the random variables that contribute to exposure. Once the mathematical model has been explained and justified, the process of determining the exposure distribution is relatively straight-forward. Nevertheless, much of the work of conducting an exposure assessment involves collection and analysis of available data for the different random variables in the model, as well as explicit representation of the assumptions about variability and/or uncertainty inputs to the model or the model itself.

5.2.6 What is the Role of Predictive Microbiology in Exposure Assessment?

The field of predictive microbiology is important to many microbial exposure assessments. This field is concerned with quantifying the dynamics of microbial populations which often depend on environmental and other biologic factors. Useful discussions on the mathematics and statistics of predictive microbiology are available (Ross and McMeekin, 1994, 2003; Haas et al., 1999; ECSCC, 2003; Vose, 2008). These references also cite seminal research in this discipline. ILSI (2010) looked at mechanisms that have an impact on physical distributions, characteristics of frequency distributions employed to model microbial distributions, and the impact of both physical and frequency distributions on illness risk and food safety management criteria. ILSI outlined six mechanisms that can impact the microbial distribution in a foodstuff: contamination, growth, death, joining, mixing, and fractionation (ILSI, 2010).

Functional relationships that describe microbial dynamics are typically of an input-output form. For example, an exponential growth model is;

$$N_t = N_0 e^{k \times t} \qquad \text{(Eq 5.6)}$$

Where N_t and N_0 are the number of microorganisms at times t and zero, respectively, and k is an exponential growth rate constant. Rearrangement of this relationship illustrates derivation of a transformation ratio for growth;

$$\varepsilon = \frac{N_t}{N_0} = e^{k \times t} \qquad \text{(Eq 5.7)}$$

If the constant, k, in the above equations is a negative value, then the same relationship can serve to predict attenuation of microorganisms in time unit t.

The exponential growth rate is only constant for particular environmental conditions. At a minimum, most exposure assessments will consider environmental conditions as variable between scenarios. In such cases, k is some function of temperature, pH, and/or other conditions (i.e., $k = f$(*environmental conditions*)). The reader should refer to the Center of Excellence in Microbiological Modeling or the FoodRisk.org[34] web sites for research regarding how various environmental factors – and microbial strain differences – influence microbial growth and/or attenuation behavior. Specific guidance for statistical fitting of experimental microbial growth or attenuation data can be found in these references.

Growth or attenuation functions can be deterministic (i.e., one set of parameters predicts one amount of change) or stochastic (i.e., one set of parameters predicts a probability distribution for amount of change). In the context of an exposure assessment, however, the predictions from either a deterministic or stochastic function will be stochastic because the environmental parameters upon which growth or attenuation depend are variables. This source of variability relates to human behaviors such as storage times and temperatures that vary across individuals.

Clearly, human behavior can be highly variable; behaviors such as exposing raw foods to high temperatures for extended periods, attenuation due to natural die-off in the environment as well as inactivation by water treatment processes can dramatically affect the dose of pathogens ultimately consumed. Although data on microbial growth or attenuation behavior can be generated readily in experimental laboratories, data on human food handling behaviors must be collected via well-designed human population surveys. Such data are available for some commodities, such as ready-to-eat foods (Kosa et al., 2007; Pouillot et al., 2010), but not necessarily for all food commodities. Although data regarding refrigeration temperatures may be applicable to most perishable foods, storage time within the refrigerator may depend on the particular food; this phenomenon

[34] http://www.foodrisk.org/resource_types/tools/predictive_micro.cfm

can only be captured via food-specific surveys. In addition, times and temperatures that foods experience during transport from retail to homes, during food preparation and prior to (or following partial) consumption are sometimes needed. Actual human behavior data that captures variability in cooking processes is also sometimes important for estimating the microbial attenuation achieved prior to consumption.

Exposure modeling allows for more complex growth and attenuation models (Baranyi and Roberts, 1994). For example, the Gompertz equation, or modifications thereof, includes specific parameters for lag time and asymptotic maximum density (Haas et al., 1999). Lag time is a characteristic of many growth curves; measured from time zero, it is the elapsed period before exponential growth begins. The maximum density that a microbe can attain before competition for nutrients halts microbial growth is another modeling characteristic studied by predictive microbiologists.

Predictive microbiology provides insight and data concerning the behavior of microorganisms across different environmental conditions. Nevertheless, such insight and data needs to be translated and extrapolated from experimental studies to natural conditions when applied to exposure assessment. Adjustment of results from controlled experimental settings to highly variable (and uncertain) natural conditions can be difficult. Therefore, care should be taken when applying predictive microbiology to exposure assessment.

Common difficulties for direct application of predictive microbiology to exposure assessment are accounting for variable temperatures across time and accounting for the competitive effects of other microorganisms on the growth characteristics of a target microbe. Varying temperatures suggest variable transitions between growth, maintenance, and attenuation of microbial populations. Depending on whether growth or attenuation is a process with memory or is memory-less, the modeling techniques needed for microbial dynamics will be different (Vose, 2008). The existence of other microorganisms in media can influence the growth rate or maximum density a target microbe can achieve, which is termed the Jameson effect (Ross and McMeekin, 2003).

Although not necessarily a part of predictive microbiology, a number of factors can affect the movement of microorganisms in the soil and potentially into groundwater, including rainfall, soil type, adsorption and desorption, surface charge of the microorganism, and pH. These processes of microbial transfer and cross-contamination also are not well researched and additional development of modeling approaches is needed for these potentially important processes. Some default techniques have been suggested for use in food safety applications (ECSCC, 2003).

5.2.7 How Can I Address Secondary Transmission of Disease in the Population?

Refer to section 4.1.5 for a parallel discussion of this topic. The approach described heretofore assumes that exposures result directly from the media of interest (e.g., food or drinking water) and the potential for person-to-person transmission of

disease is not taken into account. Such an approach generally assumes that multiple or recurring exposures constitute independent events with identical distributions of contamination (Regli et al., 1991). Furthermore, secondary transmission and immunity are assumed negligible in this approach. Nevertheless, such assumptions may not be valid.

To more completely assess all possible exposures, it may be necessary to consider possible secondary transmissions that result from a primary infection (Soller and Eisenberg, 2008). Such an approach commonly requires consideration of a disease transmission model (refer to Figure 6.2 as an illustrative example). A variety of models have 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; Riley et al., 2003; King et al., 2008). Modeling infectious disease processes such as person-to-person transmission of infection and immunity requires dynamic methods where the number of susceptible individuals is time-varying and risk is manifest at the population level (Anderson and May, 1991; Hethcote, 1976, 2000).

Epidemiological disease transmission models stratify a population of potentially exposed humans into different states according to disease status:

a) Susceptible;

b) Diseased (infectious and symptomatic);

c) Carrier (infectious but asymptomatic);

d) Immune (partial or complete).

Only a portion of the population is in the susceptible state at any point in time, and only those individuals in a susceptible state can become infected through exposure to pathogens. Members of a population may move between model states, and model parameters predict the numbers of people that are in each of the epidemiological states at any given point in time. 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). The rate parameters may be determined through literature review or through site-specific data.

Disease transmission models may also be used to determine the primary exposure and to focus explicitly on the environment (Eisenberg et al., 2002, 2005; Sheng et al., 2009). Such models may be necessary to predict the level and frequency of contaminated media when little or no empiric evidence is available. For example, assessing exposures that result from the inadvertent slaughter of a Highly Pathogenic Avian Influenza (HPAI)-infected U.S. poultry flock requires modeling the epidemic spread of the virus

within that flock (USDA, 2008a). This approach is needed because there is no evidence available concerning the occurrence of this pathogen among U.S. poultry flocks.

A more detailed treatment of infectious disease modeling and secondary transmission modeling for airborne microorganisms, such as anthrax and severe acute respiratory syndrome (SARS) virus (Riley et al., 2003; Bartrand et al., 2008; Spicknall et al. 2010; Atkinson and Wein, 2008; and Noakes et al., 2006) are not discussed here. Other considerations may also address how to interpret various types of data for use in transmission modeling. For example, although serum antibody data can be used to characterize the longevity of protection of infection, new data are appearing in the literature (for example based on salivary samples), and interpretation of those data will be needed prior to their incorporation into MRAs.

5.2.8 What Data Can I Use in an Exposure Assessment?

Ideally, the data needed for an exposure assessment are determined by the specific conceptual and mathematic models identified in the planning and scoping phase (refer to Chapter 2). If the needs are clearly determined before effort is expended in collecting and analyzing data, a fuller and more efficient treatment of relevant data can be accomplished.

Exposure assessment data usually stem from either population- or experimental-based surveys or studies. These data are preferably from published or reviewed research and are fully relevant and representative of what is needed in the exposure assessment. However, this is not always the case. An extensive discussion of data types and sources is available (FAO/WHO, 2008).

The broad categories of data needs for exposure assessment are: microorganisms, processes, and characteristics and behaviors of exposed humans. Within each of these categories, however, is an array of data types that may be needed for specific analyses. Each of these broad topics is discussed below.

<u>Data on Microorganisms</u>

Data about the occurrence and amount of microorganisms within the medium of interest (e.g., water, food, air) is important for process modeling. It is desirable to have occurrence data for multiple points between the beginning of the model and the point of exposure. For example, data on human shedding of microorganisms, transfer rates from surfaces to hands, and survivorship data may all be useful for process modeling and exposure assessment.

Prevalence data[35] provide presence/absence data for the occurrence of a microorganism in a medium. Such data support estimation of the proportion of some

[35] Note that prevalence also has a very specific and different meaning within the field of epidemiology (i.e., the number of cases of a specific disease or condition per population at a given time or age).

population in which the microbe occurs (i.e., $prevalence = \frac{\#\ of\ units\ with\ microbe}{\#\ of\ units\ in\ population}$) during some cross-section of time. Observational studies that solely report prevalence are rarely directly applicable to exposure assessments. Instead, the apparent prevalence must be adjusted for the probability that units with the microbe would be detected if actually present (i.e., sensitivity) in order to estimate the true prevalence. Apparent prevalence is also influenced by the probability that units without the microbe might be incorrectly detected (i.e., 1 – specificity).

Counts of microorganisms in sampling studies are desirable for exposure assessments. Such data may arise from microbiologic techniques such as direct plating, observation, or most probable number assays (Haas et al., 1999). These data provide an empirical distribution of the number of samples that contained each count of microbe observed. Nevertheless, it also is important to know the sensitivity and specificity of the methods used in count assays to interpret the data accurately.

A well-designed exposure assessment fully characterizes the microbe (or microorganisms) on which it is focused. Oftentimes, occurrence data will not be specific for the target microbe and will require additional data to interpret the relevance of the occurrence data. For example, count data for all *Salmonella* serotypes on broiler carcasses would need to be adjusted if the focus of the exposure assessment was *Salmonella enteric* Typhimurium. If a Poisson distribution adequately describes the count data, and the data estimated that 20% of all *Salmonella* were *Salmonella enteric* Typhimurium, then model the counts of *Salmonella enteric* Typhimurium as distributed according to a Poisson ($0.2 \times \lambda$) distribution. Yet, other data may suggest this simple approach does not adequately account for the clustered occurrence of specific *Salmonella* serotypes on broiler carcasses.

<u>Process Data</u>

General processes common to many exposure assessments include growth and attenuation of the target microbe(s), as well as mixing and partitioning of the medium in which the microbe occurs (Nauta, 2002). The evidence used to construct the conceptual model should inform the processes modeled in an exposure assessment.

Predicted changes in microbial amounts resulting from growth or attenuation processes may be available from predictive microbiology research. Nevertheless, these predictions are often functionally dependent on environmental factors. Therefore, you need data to characterize the variability in parameters such as temperature and time in order to employ predictive microbiology in an exposure assessment.

Partitioning of water, food, or air into smaller subunits prior to exposure is a common problem in exposure assessment that requires industry or ecologic data to solve. Mass balance considerations may be required to account for recycling or cross-contamination of microorganisms in some scenarios. Some of these data may come from

industry- or government-sponsored surveillance systems; but expert experience will be the only information available sometimes.

Human Characteristics and Behavioral Data

Demographic and behavior data concerning exposed humans will be specific to the populations and media considered in the exposure assessment. Much of the data used to characterize populations will come from routine government surveys. These surveys provide demographic data by geographic region, age, sex, and other factors. The estimated proportion of the population that represents a susceptible population may be available from epidemiologic research. Extrapolations from non-representative data may require substantial modeling and expert judgment to accomplish.

Data on human behaviors that influence the exposure assessment will be needed. Some behaviors, like time and temperature of storage or cooking, are highly variable among the human population. Some of these behaviors have been the subjects of on-going research projects.[36] These data inform the growth and inactivation processes via their predictive functional relationship with microbial counts.

Specific data on some human behaviors that increase the likelihood of exposure to a particular microbe (e.g., preference for raw meats or seafood, occupational exposure to microbe rich environments, cohabitation with infected individuals) are sometimes difficult to locate. Nevertheless, some frequency and contact rates have been summarized for water and air media (Haas et al., 1999). Similarly, government surveys can provide data on variability in consumption, inhalation, or contact amounts across individuals and groups of individuals.[37]

Some well known and frequently used sources of human consumption data include CDC's National Health and Nutrition Examination Survey (NHANES)[38] and USDA's Continuing Survey of Food Intakes by Individuals (CSFII), which as of 2002 have been integrated and maintain the name NHANES (Dwyer, 2003). The FoodNet Population Survey Atlas of Exposures is also a useful resource.[39]

5.2.9 How do I Use Data in an Exposure Assessment?

Data provide evidence about the inputs to the exposure assessment, but data also influence the magnitude of uncertainty surrounding its results. Weak or absent data are usually associated with large uncertainties while data that are substantial, relevant, and representative contribute little uncertainty to an exposure assessment.

Population-based, observational data are commonly used to estimate the parameters for random variables in exposure assessments. Statistically robust approaches

[36] www.cfsan.fda.gov/~lrd/ab-foodb.html
[37] www.ars.usda.gov/main
[38] http://www.cdc.gov/nchs/nhanes.htm
[39] http://www.cdc.gov/foodnet/surveys/FoodNetExposureAtlas0607_508.pdf

for selecting appropriate probability distributions, estimating the parameters of those distributions, and comparing alternative distributions are explained elsewhere (Haas et al., 1999; Vose, 2008). Often the process of fitting data to distributions is complicated because the data were generated by imperfect detection systems. Adjustments for imperfect detection sensitivity and specificity are discussed in those same references.

Although there are pros and cons to strict application of either classic statistical ("frequentist") or Bayesian estimation methods, it is often the case that the results of the two approaches are very similar. Results will tend to differ when the available dose-response data are very limited and/or when there is substantial information other than the numerical dose-response data that leads to a very informative (i.e., precise) prior.

Procedures for statistical fitting of data to distributions include appropriate methods for determining the uncertainty in the estimated distribution parameter(s). This uncertainty is propagated through the exposure assessment and combined with other sources of uncertainty to quantify the total uncertainty about the resulting exposure distribution.

Data of questionable relevance to the specific exposure assessment require special consideration. Similarly, data that are not entirely representative of the populations modeled should be carefully used. It can be argued that data pertaining to one microbe are also relevant to another. Such 'surrogate' relationships should only be exploited in the absence of data that is directly relevant and should be used in a transparent manner. Establishing the credibility of a surrogate for the target microbe may require a high standard of proof. It is usually preferred to use directly relevant data and honestly represent its uncertainty than to mix highly relevant and surrogate data in an attempt to reduce uncertainty.

Highly representative data are generated from random sampling of all relevant populations. For example, an exposure assessment pertaining to the United States would preferably use human behavior data generated from a representative sample of U.S. persons. Nevertheless, representative data may not be available for some model inputs. Instead, data from specific regions or other countries may be available. Based on comparison of other factors it may be concluded that one or more of these other data sources could be a reasonable substitute. In such cases, an effort can be made to determine the best substitute and only use its data in the exposure assessment. It is usually inappropriate to mix multiple sources of less representative data because the resulting estimates often imply more confidence than is legitimate to claim.

5.3 How do I Analyze a Model's Results?

The general purpose of an exposure assessment is to translate the technical inputs of a model into a description of the likelihood of exposure doses in some defined part of the human population. Quantitative assessments estimate numeric values while qualitative assessments may use ordinal metrics (e.g., high, medium, and low) to signify the magnitudes of exposures.

Quantitative exposure assessment models usually comprise random variables that consequently generate variability in exposures. Therefore, a common output of the exposure assessment is the frequency distribution of possible doses that come in contact with the human population of interest. This distribution is an estimate of the actual variability in exposures that occurs in nature. If the model is calculated based on input values thought to accurately reflect current conditions, this variability reflects what is occurring at present. Exposure assessments often refer to such predictions as the baseline exposure. If the model is calculated based on proposed risk management changes, then the variability reflects a prediction about the future.

It should be noted that the exposure distribution calculated from a model is also constrained by some unit of time. This time component must be explicit to correctly interpret and extrapolate the variability predicted by the model.

Analysis of the exposure distribution includes determining the sensitivity of this distribution to changes in the random variables (or other model inputs) used to predict it. Sensitivity analysis determines which model inputs are the primary drivers or predictors of substantial doses with relatively high likelihoods of occurring. Conclusions about the important inputs directly inform risk managers by suggesting what changes in the system will cause the greatest reduction in exposures.

If all model inputs were perfectly known, then the output of an exposure assessment might arguably consist of a single exposure distribution. Yet uncertainty potentially pervades all aspects of an exposure model and the resulting uncertainty about its predictions can be incorporated into any analysis. Uncertainty analysis is concerned with determining the influence of the various sources of uncertainty on the predictions of the exposure assessment.

5.3.1 How do I Report Exposure in an Exposure Assessment?

The natural output of an exposure assessment is an exposure distribution; this distribution provides likelihood or frequency values for the range of possible doses that constitute exposures. Clearly identify the applicable time period and the exposed human population to which the exposure distribution applies. The most common formats for reporting exposures are tables or graphs.

An exposure distribution may reflect the possible doses an individual could experience in, for example, one year. The objective of the risk assessment should determine the type of exposure distribution reported. It could reflect those doses relevant to a highly sensitive population, life stage, or to the entire population.

An exposure assessment will convey the variability of doses for the relevant population per unit time, but it may also include consideration of the variability of the entire distribution across time. A dynamic exposure assessment that predicts trends in exposure across time may report the trends based on the statistical expected values of the

individual exposure distributions or it may report the actual distributions for each time period considered.

It is essential to communicate the magnitude of uncertainty about the true exposure distribution. A number of techniques may be used to convey the uncertainty, but no method is universally applicable and all methods can be computationally intensive (Cullen, 1999; Lammerding and Fazil, 2000). Second-order modeling is a common technique to accomplish a clear separation of variability from uncertainty. In second-order modeling, variability is derived for one combination of uncertain inputs and the process is repeated until a full range of plausible combinations is achieved (Vose, 2008). Nevertheless, the complexity of this technique may preclude its widespread application.

Multiple exposures to various doses for the same individual can complicate exposure assessments. It is not uncommon to assume that exposure to non-zero doses is an infrequent phenomenon within a population. Therefore, a one-to-one correspondence between exposures and individuals is assumed, or at least the same individual is unlikely to be exposed twice within a short period of time. Nevertheless, in some cases this assumption is not robust, especially when considering exposures that may be clustered in space and time. Microbial dose-response models typically describe the likelihood of an adverse outcome as a function of a single exposure to some dose. If people are expected to face multiple exposures, then the process of combining the exposure information with a typical dose-response function will be different from a standard one-dose one-person approach (Haas et al., 1999).

5.3.2 How do I Determine a Change in Exposure and Subsequent Risk?

Many MRAs are used to determine how risk management decisions might change the risk of an adverse human health outcome. From the perspective of an exposure assessment, this usually requires calculating the model with and without the proposed change. For example, risk managers may want to evaluate the effect of some new mitigation process on the frequency or level of exposure doses. A baseline exposure prediction is compared to an exposure prediction based on the inclusion of the new mitigation process.

In more complex exposure models, measuring change in exposure is complicated by the role of uncertainty in the model's predictions. The baseline model predicts an exposure distribution with its attendant uncertainty. The mitigation model will predict a different exposure distribution with its own attendant uncertainty. But the uncertainties between the two predictions are not independent of each other. It should be clear that the same things that contribute uncertainty to the baseline predictions usually apply to the mitigation predictions. Therefore, measuring the change in exposure between the two predictions is not trivial. Typically, the change to be quantified is the number of adverse human health outcomes. That change cannot be calculated until risk characterization takes place and the problem of dependent uncertainties is compounded by the inclusion of dose-response uncertainty.

A direct method for measuring changes in exposures or adverse human health outcomes accurately is to model the baseline and mitigation predictions simultaneously using some parallel modeling structure. This method is computationally daunting, however, and is not commonly followed.

Another approach can provide boundaries for the magnitude of change in numbers of adverse human health outcomes. In this approach, two uncertainty distributions about numbers of adverse human health outcomes, generated by separately calculating the baseline and mitigation predictions, are subtracted from each other assuming perfect positive correlation and assuming perfect independence. Generic equations for each approach are:

Independence or perfect correlation

$$E[B-M]=E[B]-E[M] \qquad \text{(Eq 5.8)}$$

Independence

$$Variance[B-M]=Variance[B]+Variance[M] \qquad \text{(Eq 5.9)}$$

Perfect correlation

$$Variance[B-M]=Variance[B]+Variance[M]-\left(2\times\sqrt{Variance[B]}\times\sqrt{Variance[M]}\right) \qquad \text{(Eq 5.10)}$$

These equations provide the expected value and variance of the difference using the two approaches. It may be appropriate to assume some parametric distribution for change in adverse human health outcomes; in that case, these moments can be used to determine that distribution's parameters. Assuming independence and perfect correlation, the results provide boundaries for the more accurate predictions that could be generated using parallel calculation. In some cases, the bounds may not be sufficiently different to cause concern but in other cases this analysis may suggest the need to invest in the development of a parallel model structure.

5.3.3 What is Sensitivity Analysis?

Sensitivity analysis examines the relative influence and importance of a model's inputs on its output (see section 6.6). A well-designed exposure assessment model should comprise inputs that influence exposures, so the important idea of sensitivity analysis is measuring the 'relative' influence. Sensitivity analysis may be completed as part of an exposure assessment or it may be done as part of risk characterization.

There is no universal standard for conducting sensitivity analysis (Frey et al., 2003). In fact, multiple approaches may be legitimately used because each approach may examine a different type of influence. The typical sensitivity analysis examines the

magnitude of change in exposures for some change in inputs (i.e., $\frac{\Delta output}{\Delta input}$). One challenge is determining how a change in exposures should be measured. In some cases, change in the average dose value may be satisfactory (e.g., measuring conditional expected dose). In other cases, the change in the variability of doses may be of interest. If sensitivity is measured using analysis of variance techniques, then changes in the average and variance can be assessed together (Frey et al., 2004). Correlation or other quantitative measures of association (e.g., spider plots, tornado charts) are commonly available in commercial software packages used for Monte Carlo simulation (Vose, 2008). Another approach used in MRA is regional sensitivity analysis, which estimates the impact of a number of parameters contemporaneously. Feasible parameter ranges and parameter distributions are defined prior to the analysis. Then, a Monte-Carlo-Analysis sometimes combined with a Latin-Hypercube sampling scheme is used to produce a large number of different parameter combinations and to obtain a model response for each combination. These multiple model responses are than analyzed to obtain information about the impact single parameters on the model response (Spear and Hornberger, 1980; Eisenberg et al., 1996).

To proceed with sensitivity analysis, it is important that the objective of the analysis is clear to the analyst. This objective can be informed by the overall purpose of the risk assessment as defined during planning and scoping (Chapter 2). Sensitivity analysis for its own sake is seldom rewarding when applied to complex models and may not be an efficient use of the analyst's resources. For a focused exposure assessment, with specific purposes and scope, it is likely that focused analysis on the importance and influence of specific components (examined in multiple ways) may be more useful for risk managers.

The major challenge for sensitivity analysis is that it is difficult to separate sensitivity from uncertainty in most exposure assessment models. Uncertainty about model components can result in a very mixed description of the importance of model components. Theoretically, a model input may be highly influential across part of its uncertainty range but much less influential across another part. At the least, acknowledgement of such discrepancies should be communicated to risk managers. Again, focused sensitivity analysis facilitates deeper analysis of a few components instead of superficial analysis of many components.

5.3.4 What is an Uncertainty Analysis?

The goal of uncertainty analysis is to identify those model inputs for which uncertainty substantially contributes to the total uncertainty about exposures implied by the exposure model (see section 6.6). Uncertainty is a lack of knowledge; therefore, accumulation of new knowledge reduces uncertainty. Uncertainty analysis suggests where to focus future data gathering efforts and/or scientific research. Like sensitivity analysis, no standard method exists for conducting uncertainty analysis.

Although the objective of uncertainty analysis differs from sensitivity analysis, the results of uncertainty analysis are usually not independent of the results of sensitivity analysis. If uncertainty about an input contributes substantial uncertainty about the model's results, then that input usually is also likely to be identified as highly influential through sensitivity analysis.

Random variables and parameters in an exposure model are subject to parameter uncertainty (Morgan and Henrion, 1990). This source of uncertainty refers to sampling and measurement errors that are inherent to empirical data. Statistical techniques may be available to quantify parameter uncertainty.

Uncertainty about model structure is another source of uncertainty that may be propagated through an exposure assessment. This source may refer to use of surrogate variables, model simplifications or alternative specifications of the model processes. An example of the latter reference might be uncertainty about whether to include a process (e.g., cross-contamination) in an exposure model. It is often difficult to quantify the magnitude of uncertainty about model structure. Nevertheless, such uncertainty can substantially alter exposure predictions from a model. Uncertainty associated with model specification can be investigated by testing the differences in fit and predictions of multiple model forms. If the models are of the same general form (e.g., exponential family), then the effects of including or excluding covariates can be evaluated using likelihood-based criteria, such as the Akaike information criterion (Akaike, 1981).

If economic information is combined with uncertainty analysis, a value of information (VOI) analysis approach may yield insights relevant to the goal of uncertainty analysis. Yet, these methods have rarely been employed in microbial exposure assessment. For a simple dichotomous decision, value of information methods can evaluate the economic returns of hypothetical new information relative to the choice made prior to acquiring the new data. These methods highlight an important point about new information; if additional data will not change a decision, then those data are not valuable. Therefore, risk managers can acknowledge that their decisions hinge on the degree of uncertainty about model results. Newly acquired data will presumably reduce that uncertainty, but its value may be naught if risk managers were not influenced by the magnitude of uncertainty about the original results.

The techniques for uncertainty analysis are similar to those for sensitivity analysis. Factorial design is a type of experimental technique that evaluates how alternative values for uncertain model inputs influence the model results (Montgomery, 2009). For example, if there are k uncertain inputs and two realistically extreme values (i.e., high and low) for each input are proposed, then the model can be calculated 2^k times to examine the influence of each input's uncertainty on the results (ECSSC, 2003). This approach supports examining interactions between the inputs. For example, one extreme value of one input may slightly influence the model's results, but when the model is calculated with that extreme value and certain other extreme values of other inputs, the magnitude of its influence substantially increases.

Analysis of uncertainty is daunting if the number of uncertain parameters or alternative model structures is large. A simple, univariate alternative to the 2^k factorial design is to calculate results for each of the two extreme values of each input independently; this only requires 2^k re-calculations of the model (ECSCC, 2003). The predicted change in results for each input can be graphically displayed to demonstrate their relative influence on the model's results.

5.4 What Can I Put Into an Exposure Assessment Report?

Communication of exposure assessment results is challenging because the intended audience is usually diverse. A presentation of these results has to satisfy technical specialists as well as those who are less specialized in quantitative methods (ECSCC 2003; FAO/WHO, 2008). Consequently, a balance between technical details and general conclusions of the analysis is a goal of any communication (see Chapter 8).

The credibility of an exposure assessment is enhanced through peer-review and feedback from public outreach. If communication of the exposure assessment is reasonably transparent and balanced, reviewers can focus their attention on the merits of the analysis and contribute to improving its accuracy and validity.

The output of an exposure assessment is usually an exposure distribution. Thoughtful contemplation of the best formats for communicating this distribution and its uncertainty should precede any final decision. A number of graphical formats for presenting risk results are available (Vose, 2008). Tabulated results can be more useful in some cases and both table and figures may be needed in other cases. A limited number of moments (e.g., mean, variance, skewness) along with meaningful quantiles of this output are routinely provided in a report. Select the best format for displaying the data. All tables, graphs, and other figures should have clear, concise narrative text to help the reader understand what is being presented.

In the report, summarize the outputs from processes included in the exposure assessment. Some readers may find plots of the central tendencies of microbe counts that illustrate trends across the breadth of a model useful.

To clearly communicate the scenarios considered in the exposure assessment, illustrate diagrammatically the conceptual model in the report. A well-annotated conceptual model will enhance reader's understanding of the analytic approach taken.

You can list all inputs used in the model and clearly define their reference symbol and name, describe their purpose in the model, provide the probability distribution name and parameter values for random variables, and explain how uncertainty for variables and parameters was handled. Communicate important inputs by using graphical depictions of their distributions.

Include the mathematical structure of the model transparently, but concisely, in the report. This information may only be useful to a specialized audience; it is usually

appropriate to place the mathematics in an appendix. Nevertheless, this mathematical description of the model will provide the greatest opportunity for clearly explaining the exposure assessment to the specialized audience. This audience can verify the validity of the model or identify errors in its logic or assumptions.

Models are always imperfect depictions of reality and their results are always conditioned on assumptions. Inclusion of a discussion of important assumptions is necessary. A transparent treatment of assumptions improves the reader's understanding of the analysis; although some might disagree with assumptions made, it is much preferred that the reader understands the reasons for the assumptions. The strength of a model, then, is based on the strength of the justifications of its assumptions.

Results of sensitivity and uncertainty analyses are presented in tabular or graphical formats and may be required depending on the use of the risk assessment. Such tables and graphs are meaningful to both a specialized and non-specialized audience. Complex analyses that do not illuminate important conclusions will create confusion for the reader. Showing the baseline scenario as a reference point is often effective for sensitivity analyses.

Exposure assessments can generate large volumes of analytic output. Nevertheless, presentation of the exposure assessment requires deliberation by the analyst; the reader of a report expects that care is taken in what is presented and how it is presented. Avoid arbitrary decisions about the content of the report.

5.5 What are Possible Future Developments in Exposure Assessment?

The discipline of microbial exposure assessment continues to grow and evolve. Compared to more established analytic fields like economics, epidemiology, or statistics, microbial exposure assessment is still relatively new. Therefore, it is expected that methods and approaches will continue to improve.

One technique of increasing interest for exposure assessment is the use of MCMC simulation (Gelman et al., 2004). The MCMC method is based on Bayes theorem, but it is designed to solve problems that would normally be intractable using standard Monte Carlo approaches. Using MCMC methods, "prior distributions" are specified for model inputs and the empiric evidence is integrated with the model to determine the combinations of model inputs best describe—in a probabilistic sense—the empiric evidence.

Many exposure assessments are generated to guide government policy development. Traditionally, risk assessments are conducted independent of economic analyses, but risk assessment products are commonly incorporated into economic analyses conducted to support government policy (Williams and Thompson, 2004). In the future, it is expected that economic analysis will be integrated into the risk assessment to provide more accurate and useful policy information.

Within exposure assessment models, costs and tradeoffs between alternative processes could be incorporated so that their effects on the model's results were explicitly calculated. For example, it might be the case that a process with variable effectiveness is associated with extraordinarily high marginal costs at very high levels of effectiveness, but low or absent costs at moderate or low levels of effectiveness. To fully appreciate such phenomena, the exposure model needs to incorporate the economic factors, otherwise the effects of these economic factors may not be understood by risk managers.

As mentioned for uncertainty analysis, VOI methods are very useful for determining economically-efficient future data gathering efforts (Yakota and Thompson, 2003; Disney and Peters, 2003). In the future, it is expected that these methods will become standard for exposure and risk assessment analyses.

Exposure assessment models can serve as templates to incorporate new data when it becomes available. In the future, exposure assessments may be updated with data collected explicitly for this purpose. Traditionally, exposure assessments use data generated for other purposes. Risk assessors then must struggle with incorporating such data into an exposure assessment. Data collected for an exposure assessment will be better structured for that purpose. The result of using data that is "fit for purpose" is improved exposure estimates for risk management decisions.

MRA deals with many different types of problems; a number of approaches and methods may provide satisfactory solutions. Nevertheless, exposure assessment will continue to evolve towards standard approaches and methods that represent accepted defaults for certain types of problems. Increasing academic attention and scrutiny of exposure assessment will almost certainly bring greater consistency in methods. Because the field of MRA is still emerging, risk assessors should search for reasonable precedents in methodology when embarking on a new exposure assessment project. As precedents are adopted and improved, standardization of methods and models should follow.

International groups, such as the WHO/FAO and Codex Alimentarius, will continue to provoke thoughtful discussions about the most appropriate exposure assessment methods and approaches. Such discussions will contribute to improve standardization of exposure assessments in the future.

5.6 Summary

The exposure assessment component of an MRA describes the route, frequency, duration, and magnitude (amount) of exposure to a microbial hazard in a population, along with the number and characteristics of the person, population, or life stages exposed. The exposure assessment and dose-response assessment are combined in the risk characterization step where risk due to a particular exposure for a defined population and a defined hazard is described.

Exposure comprises the sources, mode, route, and extent of contact the host has with the microbial hazard(s) of concern. Microbial agents may come from more than one

source, may be transmitted via multiple routes of exposure, and may be spread via secondary transmission. Moreover, these routes of exposure may be inter-related. A number of factors define microbial exposure including the sources and pathways of exposure, environmental factors (e.g., growth and/or decline in numbers of microorganisms), and intake amounts among individuals.

Exposure assessments can be either qualitative or quantitative. A qualitative exposure assessment is based on data and information that, when considered along with expert knowledge and identification of attendant uncertainties, provides a characterization of exposure in descriptive terms (e.g., high, medium, or low). A quantitative exposure assessment provides numerical expressions of exposure, which provides the likelihood of different microbial dose amounts, as well as numerical measures of confidence about its estimates. To support better risk management decisions, objectively characterize the variability and uncertainty in the exposure assessments.

6. RISK CHARACTERIZATION

As introduced in section 1.4, risk characterization is one of the four fundamental components of risk assessment. Risk characterization is the integrating component of the risk assessment process that "characterizes" or describes and summarizes microbial health risks. It is the final integrative step of the iterative risk assessment process. An important part of risk characterization is the assessment of the data and a reiteration of the risk assessment process as appropriate. This chapter addresses what a risk assessor can do to take the pertinent elements from the previous components of the risk assessment (hazard identification, hazard characterization, dose response, and exposure assessment) and integrate them into a coherent, understandable, and informative conclusion that is useful for decision makers and stakeholders. Further, the risk characterization discusses scenario, model, parameter, data, and analysis options that risk managers should understand and consider when interpreting the results of the risk assessment. The risk characterization, in a sense, brings to full circle the initial planning and scoping for the risk assessment described in Chapter 2. The content of the risk characterization is intended to reflect the issues and questions detailed during planning and scoping.

For further detail and discussion on risk characterization, refer to the NRC reports (NRC, 1983, 1994, 1996, 2009), EPA's *Risk Characterization Handbook* (EPA, 2000a), *An Examination of EPA Risk Assessment Principles and Practices* (EPA, 2004a), and material from FAO/WHO (Codex, 1999) for reference. This chapter is not intended to provide the level of detail about risk characterization provided in these references, but rather provides the microbial risk assessor with guidance on what information to include and how to integrate the information from the previous three chapters.

6.1 What is Risk Characterization?

In its most general sense, risk is the possibility (and if estimated, probability) of suffering harm. For the purposes of MRA, hazard may be causal or associated with adverse outcome as a representation of intrinsic effects expressed by a microbe. Risk contains elements of both hazard and exposure. Thus, risk is generally understood to be the integration of intrinsic effects, represented by Hazard, and the values for Exposure. Risk is usually represented by some form of the following basic equation.

$$Risk = f_{Hazard} \cdot f_{Exposure} \qquad \text{(Eq. 6.1)}$$

Hazard identification allows you to select and focus on specific features of subject organisms associated with the potential to cause harm. Exposure analysis provides a description of the routes and an estimate of the degree to which a host may be exposed. When combined with host factors and an evaluation of dose response, one can obtain a quantitative hazard characterization of that potential once a host is exposed. Risk characterization takes the specific identified hazards, examines the probabilities of their existence under specific exposure scenarios, and combines these probabilities with those

of the likelihood that the agent will encounter the host in sufficient quantity to cause an effect.

Risk characterization is the final step of the MRA process in which all preceding data collection and analyses are combined to convey the overall conclusions about potential risk to humans. During risk characterization, the results of the risk assessment process are integrated and documented in a descriptive risk characterization summary. Risk characterization communicates the key findings and the strengths and weaknesses of the assessment through a conscious and deliberate transparent effort to bring all the important considerations about risk into an integrated analysis by being objective, transparent, clear, consistent, and reasonable (OMB, 2007b; EPA 2002b; EPA 2000a). For these reasons, the risk characterization needs to be complete, transparent, informative, and useful for decision-makers. Therefore, this section of the risk assessment needs to be both sufficiently technical to be accurate scientifically, taking into consideration the uncertainties and reporting the assumptions but also comprehensible by an educated lay audience. This component most directly leads to a regulatory/management decision and serves as a communication tool for stakeholders.

Risk characterization describes the ways in which exposure and dose response (quantitative) or exposure and hazard assessment (qualitative) are integrated to formulate a statement of risk. Risk characterization can be quantitative, when values are available for all terms in the risk equation, or it may be semiquantitative, when only some values are available. In many cases, default values/assumptions based on known conditions are used in place of measured ones. Further, when the data do not adequately support a quantitative estimation of risk, then a qualitative description of the risk may be all that can be presented in a risk characterization, which may be sufficient in certain cases. Regardless of quantitative versus qualitative, the risk characterization should address the risk management questions posed in the planning and scoping phase and any questions that may have been added or revised during the assessment itself.

Risk characterization brings the planning and scoping into focus and forms the starting point for formulating risk management considerations. In addition, risk characterization provides a foundation for (regulatory) decision-making. Both quantitative data and qualitative information are characterized in technical and non-technical terms; and the extent and weight of evidence, results, and major points of interpretation and rationale are all explained. Risk characterizations also include summaries of the strengths and weaknesses of the evidence, conclusions, uncertainties, variability, potential impact of alternative assumptions, and discussions of the scenario, model, parameter, and analysis options that may deserve further consideration as the results from the assessment are subsequently used for decision making purposes.

6.2 What are the Elements in a Risk Characterization?

During the risk assessment process, you should have identified areas where policy options were considered, where management decisions and assumptions were made, and where uncertainties are important. The point of risk characterization is not to reiterate the

details of each chapter of the risk assessment, but rather to integrate those chapters to arrive at the risk assessment output (e.g., risk estimate, risk ranking, or other output), describe the relevant findings, cross-reference the exposure and dose-response assumptions (e.g., do the age groupings in exposure assessment and dose-response assessment match), and discuss other salient elements (as described below).

Risk characterization consists of two principal steps—risk estimation and risk description (ILSI, 1996, 2000). 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. The risk estimation is derived from the output components of the risk assessment (e.g., hazard identification, hazard characterization, exposure assessment, and dose-response analysis). 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. Resultant estimates of the potential for adverse human health effects 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).

Risk description puts the risk estimation into context by summarizing the event of interest (i.e., nature, severity, and consequences) and discussing and quantifying (to the extent possible) (1) the uncertainties associated with the 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; (5) the critical assumptions; and (6) the plausibility of the results. Many of the elements of the risk description stem from the planning and scoping phase. In some ways, the risk description is similar to the “Discussion” section of a scientific paper and should close the loop on the issues that were raised in the planning and scoping phase. Clearly, use your professional judgment to determine what should be included in the risk characterization.

Consider the following elements in risk characterization (adapted from the EPA *Risk Characterization Handbook*, EPA 2000a):

a) **Key information** – Consider: 1) the studies available and their robustness; 2) the major risk estimates calculated the assumptions and the extrapolations made during the estimated risk calculation, and the residual uncertainties; 3) the use of default parameter values, policy choices, and risk management decisions made, if any; 4) whether the key data used for the assessment are considered experimental, state-of-the art, or generally accepted scientific knowledge; and 5) variability.

b) **Context –** Consider: 1) how to address the risk management questions; and 2) how the estimated risk from this microbial hazard compares to other estimates for this hazard, if available. Include discussion of regulatory requirements or if there are regulatory values to consider.

c) **Sensitive Populations –** Consider: 1) the range of people that may be affected, including innately susceptible populations (e.g., ethnic groups, gender, socioeconomic and/or nutritional status, other genetic predisposition) and those that are highly exposed; and 2) a quantitative characterization for each sensitive population may not be necessary or possible.

   For example, it may be sufficient to estimate risks for the most sensitive group, and then assume that that the other groups are protected. If the quantitative portion of the risk assessment is strongest for the general population due to data availability, then some data based adjustment for sensitive populations may be considered. Both results can be presented and discussed.

d) **Life Stages –** Consider: 1) the age groupings evaluated; and 2) life stages that may have particular vulnerability due to behaviors or situations that influence exposure patterns and/or innate susceptibilities.

   For microbial hazards with only short-term effects, the different life stages may be treated as sensitive populations. If any long-term effects (e.g., health endpoints that span a 70 year life) are of interest, then life stages may need to be considered differently than sensitive populations. For example, everyone in the general population passes through childhood life stages and exposure to pathogens could vary at different childhood life stages. Thus, depending on the scope of the risk assessment, consideration of childhood life stages may be necessary as risk estimates may vary for the range of important life stages.

e) **Scientific Assumptions –** Describe: 1) where key data gaps exist; 2) what are the key assumptions used during the assessment; and 3) how the assumptions impact the assessment outcome. Also, note precedent in other risk assessments for the approach or assumptions employed, and note the justifications for selection of any default parameter values that are used.

f) **Policy Choices –** Describe: 1) if your office has different policies about how to assess risk (e.g., different uncertainty factors or different levels of regulatory concern); and 2) if any policy choices bound the scope of the assessment. If appropriate, include discussion of consistency with other agency approaches or decisions.

g) **Variability** – Describe: 1) how variability arises from true heterogeneity in characteristics such as dose-response differences within a population or differences in microbial levels in the environment; and 2) if the values of some variables used in an assessment can change with time and space (such as seasonal differences) or across the population whose exposure is being estimated.

The discussion of variability should link to the discussion of assumptions, because variability considerations may be lost or assumed when values for parameters are selected. This element is critical and discussed at length in the previous chapters. An inadequate discussion of variability can result in a loss of transparency or in the worse case a misleading risk assessment.

h) **Uncertainty** – Describe: 1) what uncertainties exist in the assessment (e.g., measurement uncertainty, model uncertainty, uncertainty due to data gaps); 2) how uncertainty is addressed (e.g., uncertainty analysis, sensitivity analysis); 3) what impact reducing scientific uncertainties could have on your assessment; and 4) where could quantitative uncertainty analyses of the data be presented,

At a minimum, a qualitative discussion of the important uncertainties should be provided. This element is critical and discussed at length in the previous chapters. As with variability, an inadequate discussion of uncertainty can result in a loss of transparency or in the worse case a misleading risk assessment. In practice, it is often difficult to separate variability and uncertainty, because many uncertainties are in the area of characterization of variability (e.g., lack of data for certain conditions). In cases where there is uncertainty about variability, be sure to be clear about your use of the two terms.

i) **Bias and Perspective** – Consider: 1) how a risk management decision, despite uncertainty and default choices, offers the direction for more public health protection compared to less protection; and 2) the potential bias that could impact the assessment so it will not be overlooked or misinterpreted by the risk manager. For example, explain the implications of selecting a 50th versus 95th percentile in a data set.

j) **Strengths and Weaknesses** – Throughout the risk characterization highlight and/or describe: 1) major imbalances among the components of the assessment (e.g., the case for the microbe posing a hazard may be strong, while the overall assessment of risk is weak because there are no data about whether there is exposure to the microbe); and 2) the strongest and weakest evidence for the conclusions. In addition, discuss the quality of the data used and how the data quality pertains to variability and uncertainty.

k) **Key Conclusions** – Describe: 1) the key points that need to be communicated for knowledgeable interpretation of the risk assessment; and 2) the small subset of key findings that support information (i.e., strengths and weaknesses, results from sensitivity/uncertainty analyses) that really makes a difference in the assessment outcome.

l) **Alternatives Considered** – Consider: 1) if there are plausible alternatives to the risk estimated in the assessment and how to deal with the alternatives (e.g., alternative models that could be used, different hazard pathways); 2) the limitations of making comparisons among the alternatives; and 3) where appropriate, how the conclusion about risk compares to other possible risks. If other risks are compared, the discussion should highlight the limitations of such comparisons as well as the relevance of the comparisons.

m) **Research Needs** – Describe: 1) the key data needs; and/or 2) methodology gaps that were identified during the course of the risk assessment.

Each element described above is important in a risk characterization; however, no single element is necessarily more "critical" than another. As the risk assessor, be aware of all these elements and address them appropriately in the risk characterization. For each element, describe the data, or in the absence of data or information for a particular element, the default assumption used.

6.3 How Do I Prepare a Risk Characterization?

The purposes of MRA vary, and the mechanisms of risk characterization, including risk integration, can also be quite varied. Some organizations prefer to attempt separate hazard and exposure characterizations, and combine the results once all the pieces are described and quantified. Others assessors prefer to have feedback from the major assessment components throughout the assessment timeframe, and thus have some elements of risk integration running simultaneously with the component assessments. Hybrids of these approaches are possible, as well. The selected characterization and integration processes may be driven by institutional practices, completeness of data, and/or timing of availability of data.

It is common to have an iterative process in which a screening characterization (e.g., risk profile) is generated early in the process. This process allows the assessors to identify data gaps, to recommend additional data generation or gathering, or to use default assumptions as a starting point to evaluate the potential implications of the scenarios being evaluated. Later iterations may adjust the scope of the characterization by focusing in on the most important hazards or exposures identified in the initial iterations. In addition, the assessment may be expanded when additional hazard or exposure elements are identified or additional data are provided.

As discussed in section 1.9 and Chapter 7, many different levels of decisions need to be made during a risk assessment. As the assessor, you take responsibility for

decisions involving scientific judgment. Other decisions that are ultimately policy calls are informed by science but will most likely be made by risk managers. Risk managers may want to evaluate policy decisions and make revisions during different iterations of the risk assessment. Any changes in policy decisions or scope can be tracked for transparency. It is unlikely that scientific judgments will change unless more data become available or compelling scientific arguments are made during internal or external peer review. It is not unusual for risk managers to adjust policy decisions or refine the scope or questions for the risk assessment during the risk assessment process. In many ways, this process highlights the importance of the iterative nature of risk assessment. You should be prepared for these types of changes and provide clear documentation to help delineate scientific judgment from policy decisions. Very often something that could be scientific judgment if data were available becomes a policy decision when data are lacking. Determining the line between those two situations can be unclear.

The level of variation in performing risk characterization is affected by available resources, time constraints, legal requirements, and agency culture. In some cases, a first attempt at a complete risk characterization, in the sense that all evaluation elements are accounted for to at least a limited extent, is desirable to provide managers with an initial scoping of likely outcomes before all information has been fully evaluated. Sometimes, certain factors are given precedence at the outset of an assessment, such as a particular exposure scenario, and interchange between the hazard and exposure components can take place throughout the course of an assessment. This scenario occurs if the examination of a specific health effect or exposure model is the driving force behind the risk assessment. In other cases, it may be necessary to isolate the hazard and exposure components to avoid results from one having an undue influence on the assessment of the other. This situation may happen if the type of assessment is intentionally broad and the desire is to not eliminate scenarios or emphasize specific effects until a first pass is made. This process can result in an iterative assessment with feedback among the components occurring during later iterations.

In selecting an approach for risk characterization, be careful to ensure that any simplifying assumptions that are employed are in fact appropriate and transparently identified. Within that context and to the extent possible, demand higher quality input data and fewer simplifying assumptions when seeking increased accuracy and precision from the risk assessment. From a modeling perspective, biological “realism” is often counter-balanced by analytical or computational complexity. The increase in the complexity of a model structure can increase variability and/or uncertainty due to increased needs associated with model specification (EPA, 2004b). On the other hand, a simpler model involves implicit or explicit assumptions that may or may not be realistic or appropriate for a particular situation. 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). As discussed in Chapter 5, statistical methods, such as the Akaike Information Criterion or similar likelihood-based measures, are used to judge the desirable level of complexity in statistical models. Representative MRA model forms are discussed in section 6.5.

Agencies may have different histories of involving economists in risk assessment. For example, EPA has recognized for many years the need for early involvement of agency economists in risk assessments, particularly when administering laws that stipulate risks and benefits analyses. Without this early involvement, outcome measures may not be useful in cost benefit analysis. Economists are trained to model and estimate firms' or individuals' responses to economic and social conditions that may result from different scenarios. The inclusion of economists on risk assessment teams as scientific analysts, not as managers, could improve the accuracy of risk assessments by incorporating models of production systems and human behavior that influence risk levels (Williams and Thompson, 2004).

6.4 Are All Risk Characterizations Quantitative, and What Do I Do When Quantitative Data are Unavailable for Some Elements of the Risk Characterization?

While many assessments are quantitative, risk characterization can also be qualitative and/or descriptive. It also is possible that some parts of the analysis can be quantitative, while only approximations and/or defaults are possible for other elements (this is sometime referred to as a semi-quantitative analysis).

Although "risk" is often thought to imply a probability of an adverse health effect and considered quantitative, some assessments can only be conducted qualitatively. In certain cases, the risk characterization can be a screening exercise, providing only a sense of whether the risk might be judged high, medium, or low. Sometimes one can obtain values for some of the components of hazard and exposure characterization, but not all. In these cases, default values may provide the missing data elements to establish limits of risk for the organism and conditions in question. A bounding analysis may be appropriate. For example, you can do a deterministic analysis with plausibly conservative values for the unknown parameters and see if the resultant risk is above the level of concern. If it is not above the level of concern, no further data on these parameters is probably necessary. If the risk is above the level of concern, then data on the unknown parameter value(s) would be justified.

Finally, a relative risk assessment may be qualitative. These types of assessment have been valuable for numerous agencies, especially for evaluating the potential benefits of management actions (treatments) or alternatives in conditions where rigorous and quantitative data were not available.

6.5 Are There Different Forms of Risk Characterization? When Do I Apply Them?

Risk characterization should be consistent with the planning and scoping process, complete, informative, and useful for decision-makers. The appropriate level of detail for any particular assessment will be a function of the goals of the assessment, the questions that the assessment are intended to answer, and the data that are available to conduct the

assessment. Whether or not a particular level of detail is appropriate for a particular situation will depend on the purpose of the assessment.

A quantitative risk characterization may be in order when hazards are well defined and specific exposure scenarios allow accurate exposure and dose-response calculations. This process is generally the case with event-driven retrospective assessments. Prospective assessments often lack sufficient information and data to make the appropriate calculations for detailed quantitative assessments. In some cases, default assumptions and/or parameter values can substitute for measured values to perform calculations with considerable uncertainty. Qualitative risk characterization may be useful when risk management choices need to be made and a general sense of risk is all that is required. For example, if default values, coupled with an understanding of the uncertainty that accompanies their use, can enable completion of a "semi-quantitative assessment" that gives enough information for decision making, then that assessment is more useful than the failure to produce any assessment, due to the inability to cope with the lack of data for which the default assumptions substitute.

The availability of data and the appropriate type of risk characterization are generally related. Assessments of specific events can sometimes result in precise calculations of exposure and dose response, assuming the agent is well identified. In some prospective analyses, estimates of organism concentrations in a particular source may be easier to estimate than the actual exposure of particular populations. In these cases, default values may need to be substituted for accurate population estimates. Similarly, dose-response relationships are only available for a limited range of organisms (refer to dose-response chapter for a list of available dose-response relationships), so quantitative assessments can only be conducted for those microbes that have known (or derivable) dose-response relationships (although those microbes can sometimes be used as surrogate or reference pathogens for other pathogens of potential concern).

With respect to quantitative risk characterization, employ a variety of model forms for the assessment of infectious disease transmission and the potential impact or benefit of intervention efforts/management actions. Particular characteristics of each model allow for the capture of different aspects of the disease transmission system (EPA, 2004a). The two most commonly employed classes of MRA models are static and dynamic models. Soller and Eisenberg (2008) provide an overview of these models along with pros and cons of each. Exclusion from the following discussion does not preclude use of a particular model form; however, justification for use of a particular model form should be included in the risk description.

Finally, the appropriate form of risk characterization may change during the risk assessment, if questions to be answered change or if additional data become available. For examples of risk characterization within MRA, refer to Foodrisk.org for a searchable database of MRAs.[40]

[40] http://foodrisk.org/risk_analysis/RA/RAs.cfm

6.5.1 When is a Static Model Appropriate?

A static model is appropriate when secondary transmission rates are negligible and the central question is concerned with the probability of infection or illness relative to the dose of pathogens acquired from a single exposure event. 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).

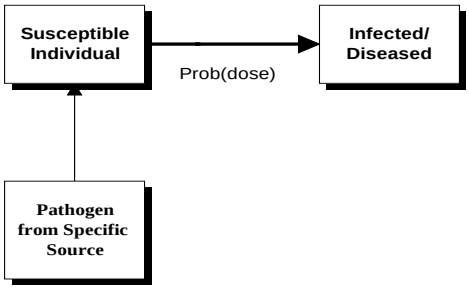

Figure 6.1 Static Risk Assessment Conceptual Model

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., *L. monocytogenes* from food, *Naeglaria fowleri* infection from water). 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 static models (parallel to those used for toxicological risk assessments) (Figure 6.1).

These models, which are based on a chemical risk assessment paradigm (NRC, 1983), are used to estimate risk at an individual level and typically focus on estimating the probability of infection or disease to an individual resulting from a single exposure event. With respect to microbial contaminants in the environment or a particular media (food, 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. Generally, but not exclusively, secondary transmission increases the level of infection/disease in a community relative to a specific exposure to

pathogens, and immunity decreases the level of infection/disease in a community relative to a specific exposure to pathogens.

6.5.2 When is a Dynamic Model Appropriate?

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 the environment or a particular medium is 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, consider addressing 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 should explore the relative importance of secondary transmission and immunity, and thus capture and integrate the dynamic interplay of hosts, agents, and environments.

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. Deterministic dynamic MRA models are suitable for large populations of individuals randomly interacting with one another. In dynamic models, the population is divided into epidemiological states such as: (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. A representative conceptual model for this type of MRA model is presented in Figure 6.2. This figure can be generalized to organisms with very short or no immunity by allowing the duration of incubation ($1/\gamma$) to approach zero.

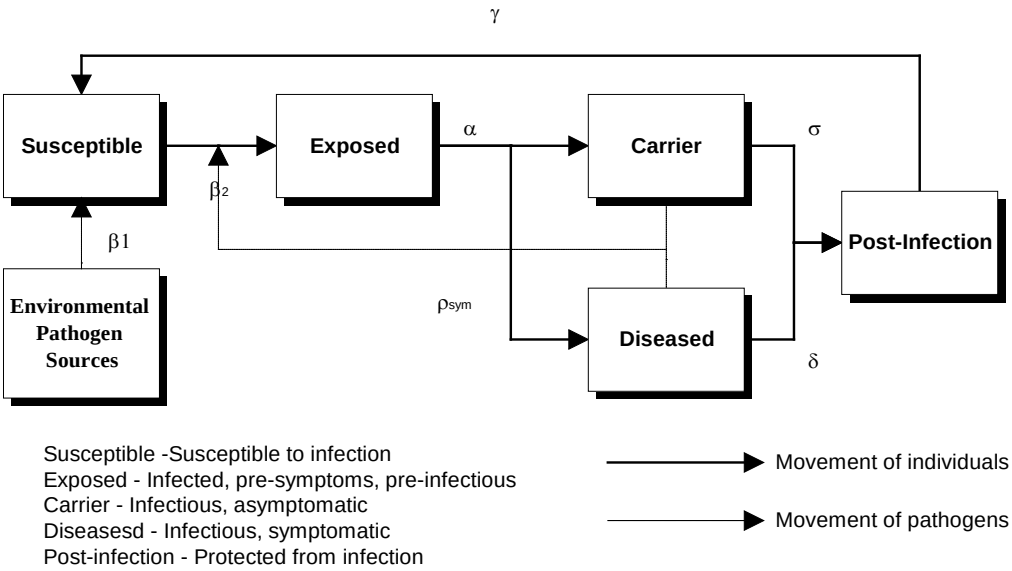

Figure 6.2 Dynamic Risk Assessment Conceptual Model
(Source: Soller, 2009; Soller and Eisenberg, 2008)

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. Deterministic dynamic MRA models have a number of limitations. For 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, and some of this 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, 2004a).

In stochastic dynamic MRA models, events are treated as random (stochastic) rather than deterministic. These models employ distributions of outcomes rather than the average outcomes of the deterministic models; a stochastic model will produce different results each time it is run. 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, 2004a).

Based on this information, risk characterization can be thought of as modular, with different modules requiring specific data for calculations. Some modules require detailed data, while others may only require default estimates or descriptive information.

Risk management options may obviate the need for precise data on exposure components, if exposure can be limited by specific actions. Thus, the appropriate module (and corresponding level of detail) for any particular assessment will be driven by the goals of the assessment, the questions that the assessment are intended to answer, and the data that are available to conduct the assessment.

6.6 How are Sensitivity and Uncertainty Analyses Related to Risk Characterization?

The discussion in this section is limited to data sensitivity and uncertainty as they relate to risk characterization and does not include sensitivity and uncertainty in overall decision-making, which may consider decision-maker judgments and values beyond the risk assessment.

Although uncertainty and variability are different in practice, it can be difficult to separate the two, particularly when uncertainty about variability is important (section 6.2). It may be practical to characterize uncertainty and variability together if clearly described. 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). Uncertainty analysis is a key concern for risk managers because it 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 should be presented clearly; in addition, their implications should be communicated clearly. Clear graphical or tabular presentations are very useful. Include intermediate calculations to the extent that they add value and understanding to the results. 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.

It also is important to carefully evaluate the impact of known sources of variability in model outputs using 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 analysis 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. Sensitivity analysis also can help determine whether more resources should be put into parameter estimation. The specific approach that is taken will depend on the nature of the data and models supporting a given assessment. USDA identified several sensitivity analytical techniques useful for MRA (Frey et al., 2004). The methods evaluated ranged 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 one or more 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 should be interpreted cautiously (EPA, 2011b). If the variations in parameter values are independent of one another, it is easy to overestimate the impact of varying more than one value; 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.

The EPA Exposure Factors Handbook (EPA, 2011b) provides several approaches to quantitative uncertainty and sensitivity analysis (Table 6.1):

Table 6.1 Approaches to Sensitivity and Uncertainty Analysis Recommended in EPA's Exposure Factors Handbook (Source: EPA, 2011b)

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

In addition, Morgan and Henrion (1990) discuss in detail the following four techniques for sensitivity and uncertainty analysis, including:

a) **Deterministic** – One-at-a-time analysis of each factor holding all others constant at nominal values;

b) **Deterministic joint analysis** – Changing the value of more than one factor at a time;

c) **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;

d) **Probabilistic analysis** – Using correlation, rank correlation, regression, or other means to examine how much of the uncertainty in conclusions is attributable to which inputs.

6.7 How are Quality of Life Measures Important in MRA?

Quality of life measures are usually included in cost-effectiveness analyses (CEA) rather than within risk assessment. You should be aware of how your risk assessment results might be used, such as in a CEA. For example, EPA has used quality-adjusted life years (QALY) and Morbidity Inclusive Life Years (MILYs)[41] in the regulatory impact analysis for the Final Clean Air Interstate Rule (EPA, 2005b, Appendix G) and the LT2ESWTR (EPA, 2006a, Appendix U).

Quality of life captures the impact of illness on 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 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. Disability adjusted life-years (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 (Gold et al., 2002; Rice et al., 2006; Airoldi and Morton, 2009).

It is important to note that QALYs and DALYs are not objective measures and require a descriptive conceptualization of present and future health states. In addition, there can be significant differences in ranking due to ethnicity, gender, and area of

[41] 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).

residence (different cities; urban versus rural) and issues around the discounting of future disease versus the avoidance of disease. 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).

6.8 How Can a Risk Assessment be Validated?

Validation and verification are two important terms for models. Verification is concerned with building the ***model correctly***. It is utilized in the comparison of the conceptual model to the computer simulation using the model. Verification asks the questions: Is the model implemented correctly in the computer? Are the input parameters and logical structure of the model correctly represented?

Validation is concerned with building the ***right model***. It is utilized to determine that a model is an accurate representation of the real system. Validation is usually achieved through the calibration of the model, an iterative process of comparing the model to actual system behavior and using the discrepancies between the two; the insights gained improve the model. This process is repeated until model accuracy is judged acceptable.

Although risk assessments can never be validated in the true sense, validation of an assessment can occur at multiple levels (Orekes et al., 1994; ECSCC, 2003; FAO/WHO, 2008). Validation of the conceptual and mathematical models, the computer algorithm and the assessment's predictions all occur before a risk assessment can be considered validated. Except for the final level, the validation process is often accomplished outside the risk assessment project.

Model validation and verification in risk assessment are general terms that are sometimes used to refer to rigorous data driven evaluation of models. 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. Verification and validation can also be defined as follows (Oscar, 2005): "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)."

The iterative nature of most assessments suggests that the models have been reviewed several times by risk managers and/or experts at the conceptual and mathematical development phases of the project. Nevertheless, public and peer review is usually solicited to examine the results of the assessment. Close scrutiny of the conceptual and mathematical models and the computer algorithm by specialists knowledgeable in statistics, epidemiology, and mathematics will serve to sanction the mechanics of an assessment model.

The output of an exposure or dose-response assessment (a dose-response relationship, an exposure distribution) is often not readily measured in nature. Surveillance data may be available for some outputs of the models, and statistical measures of agreement between the model's predictions and empirical observations are helpful in describing the accuracy of the model. Creative uses of empiric evidence may serve to support a contention of validity. Nevertheless, most risk assessments cannot meet this burden of proof concerning their validity. Such is the nature of many risk assessment problems; their verification primarily stems from the logic and reasoning built into the models used to solve them.

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. The following are illustrative examples of such comparisons:

a) **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). 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); however, 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 approximately 23% of the population became ill from rotavirus exposure during this event. The results of a 5,000 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.

b) ***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 provided 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).

Whether or not formal validation is possible, peer review is an important aspect of evaluating models (OMB, 2004).

6.9 Summary

Risk characterization is the integrating component of the risk assessment process that describes and summarizes (characterizes) microbial health risks. It is the final integrative step of the iterative risk assessment process. The risk characterization step brings the planning and scoping into focus and forms the starting point for formulating risk management considerations, providing a foundation for (regulatory) decision-making.

Risk characterization describes the ways in which exposure and dose response (quantitative) or exposure and hazard assessment (qualitative) are used together to formulate an estimate of risk. Risk characterization can be quantitative, when values are available for all terms in the risk equation. It also may be semi-quantitative, when only some values are available. Risk characterization consists of two principal 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. Risk description puts the risk estimation into context by summarizing the event of interest according to its nature, severity, and consequences.

Risk characterization should be consistent with the planning and scoping process, complete, informative, and useful for decision-makers. The appropriate level of detail for any particular assessment will be a function of the goals of the assessment, the questions that the assessment are intended to answer, and the data that are available to conduct the assessment.

7. RISK MANAGEMENT

7.1 What is Risk Management?

Risk management is a ubiquitous term used in settings as diverse as financial investing, military planning and public health. Within federal public health agencies, risk management refers to activities ranging from high-level policy making to routine, sometimes *pro forma*, risk control applications in operational risk management. This chapter provides an overview of the risk management processes likely to be encountered by microbial risk assessors.

The NRC "Red Book" initially defined risk management in very broad terms as "the process of evaluating alternative regulatory options and selecting among them" (NRC, 1983). In 1996, a subsequent NRC committee described the activities of risk managers:

> Risk managers are supposed to deal with broad social, economic, ethical, and political issues in choosing from among a set of decision options by using the results of the risk assessment and their understanding of the other issues. Making tradeoffs, which may be called risk-benefit, cost-benefit, or risk-risk evaluations, is part of risk management. (NRC, 1996)

The NRC reports focused on management processes occurring with a single risk assessment. Concurrently during the mid-1990s, the Presidential/ Congressional Commission on Risk Assessment and Risk Management argued that risk management should no longer be thought of a process that focuses on decisions about managing one risk at a time. Rather, governmental agencies need to confront the task of managing risks from multiple hazards and exposures. To this end, the Commission provided a framework for risk management (Text Box 7.1; P/CC, 1997).

Text Box 7.1
Presidential/Congressional Commission on Risk Assessment and Risk Management – steps to include in risk management

- Formulate the problem in broad context
- Analyze the risks
- Define the options
- Make sound decisions
- Take actions to implement the decisions
- Perform an evaluation of the effectiveness of the actions taken

The engineering and systems analysis view of risk management has been described by Haimes (2004). Risk management is the process focused on controlling risks by addressing:

a) What *can be done* and what are the *options* for controlling risks?

b) What are the *trade-offs* in terms of risks, benefits and costs?

c) What are the *impacts of risk management decisions* on future options for risk management?

Federal state and local public health agencies are charged with the responsibility of preventing, mitigating, or controlling risks to the public's health. As a general concept, it is clear that the "mission" of risk management is accomplished using risk management processes at several levels. For example, Table 7.1 describes risk management as a strategic, applied, or operational function in the agency. The strategic level is concerned with managing the agency's portfolio of risks; the applied level—the primary focus of this guideline—concerns the risk management processes surrounding specific risk assessments; and operational risk management deals with risk management that is guided by standard operating procedures.

Table 7.1 Classes of Risk Management in Federal Agencies

Class of Risk Management	Description
Strategic Risk Management (and Policy Making)	Long-term, broadly based view of the agency's entire risk portfolio. Interface with the public, industry and governmental stakeholders about policy issues such as the level of acceptable risk, the risk-based decision- making process.
Applied Risk Management	Charters and collaborates with risk assessors on newly identified or emerging risks, new scenarios for known risks and risk mitigation scenarios. The information gained from the risk assessment is used in risk management decisions about controlling the risk.
Operational Risk Management	Implements prescribed administrative, engineering or other controls to maintain risk at appropriate levels or below.

The Codex *Principles of and Guidelines for the Conduct of Microbial Risk Management* (MRM) includes the following 8 principles (Codex, 2007a):

a) Principle 1: Protection of human health is the primary objective in MRM.

b) Principle 2: MRM should take into account the whole food chain.

c) Principle 3: MRM should follow a structured approach.

d) Principle 4: MRM process should be transparent, consistent and fully documented.

e) Principle 5: Risk managers should ensure effective consultations with relevant interested parties.

f) Principle 6: Risk managers should ensure effective interaction with risk assessors.

g) Principle 7: Risk managers should take account of risks resulting from regional differences in hazards in the food chain and regional differences in available risk management options.

h) Principle 8: MRM decisions should be subject to monitoring and review and, if necessary, revision.

7.2 When and How Can Risk Managers be Involved in Risk Assessments?

Risk management begins before risk assessment. Sometimes the recognition of a potential problem and the general hazard identification occurs externally to the agency and is brought to the attention of risk managers by the public, stakeholders, or other governmental organizations. Risk managers typically determine the need for a risk assessment and provide the risk assessment team with the specific risk analysis to be performed. This often includes setting the analytical boundaries and constraints for the risk analysis. For example, the risk assessment might be focused on risks from exposures to the hazard only within the U.S. borders or, perhaps to a particular population at risk of illness from exposures to the hazard. During planning and scoping, the initial problem formulation and discussion about boundary conditioning are often accomplished interactively with risk assessors who have the particular knowledge about what can be performed quantitatively and whether or not a quantitative risk assessment can be accomplished within the project time constraints. Because risk assessment is an iterative process, risk managers are involved in helping scope the different iterations.

Risk managers should work interactively with you (risk assessors) during the planning and scoping activities to collaborate on defining clear, scientifically defensible "risk questions" before the analytical components of risk assessment are executed (FDA, 2002; Dennis et al., 2008; NRC, 2009). Forming a risk question is analogous to stating a testable hypothesis at the outset of a basic research project; it is a necessary antecedent to designing an objective and informative project. Risk managers are generally aware of the type of information needed to answer policy questions, the resources available to mount complex and large-scale risk assessments, and relevant stakeholder concerns. You will probably rely on risk managers for a "big picture" perspective of the agency's entire portfolio of risk management activities and how the current risk assessment fits into the agency's work plan.

Risk managers have valuable insights into the value and potential problems of risk assessments. Thus, high quality risk management requires risk managers to interface with you at various stages throughout the entire risk assessment process so that they can help you anticipate problems in the analyses and redirect resources, if necessary, to improve or ensure the quality of information resulting from the risk assessment. Additionally, risk managers might become aware of new information about the risk in question that might be useful for focusing the risk assessment on a modified risk

question. The frequency of risk assessor and risk manager discussions will likely depend on the complexity and nature of the risk being investigated.

The risk managers should explain clearly why the assessment is being performed and what questions need to be addressed. The risk managers should also advise the assessors, economists, engineers, and other contributing experts involved in the planning and scoping of any interested party, affected party, or policy interests to be considered in the context of the risk issue. These factors may influence the risk management options, management goals, key participants, data sources, selection of assessment endpoints, or the schedule for the development of the assessment. The risk manager and appropriate others should discuss any regulatory basis for the risk assessment and what kind of information is required to satisfy such requirements.

Risk assessment teams usually have a lead risk assessor.[42] The lead risk assessor is responsible for ensuring that risk assessments are properly performed and documented and that the key information from risk characterization is elevated up the management chain and communicated to senior management. The lead risk assessor should ensure that the risk characterization integrates other considerations specified in applicable statutes, agency and office policies, executive orders, and other factors to make and justify regulatory decisions. The lead risk assessor's specific responsibilities might include:

a) Ensuring that all risk assessment work products produced by or submitted to your organization are well written and characterized.

b) Providing advice, guidance, and support for the preparation, conduct, and completion of an appropriate risk assessment for your decision.

c) Playing a major role in managing and documenting the planning and scoping process.

d) Ensuring that sufficient funds are designated in the office's budget request to conduct a risk assessment.

e) Establishing a realistic risk assessment schedule.

f) Ensuring that the products prepared by individual risk assessors for their portion of each risk assessment document are integrated into a complete risk assessment.

g) Establishing systems to maintain records of the risk assessments prepared by risk assessors under your supervision.

h) Ensuring that the key points from the risk assessment are carried forward in all deliberations or considerations for decision making.

[42] Synonyms for this position include, technical integrator, risk assessment team leader, risk assessment team liaison, and risk assessment manager.

i) Reviewing implemented decisions for the degree of implementation, efficacy, and ongoing relevance.

j) Ensuring that the uncertainties and their implications are communicated to the risk managers in lay terms.

7.3 How are Risk Management Options a Useful Component to Include in a Risk Assessment?

"Risk managers use information from risk assessment and economic analysis, together with information about public values and statutory requirements, to make decisions about the need for and methods of risk reduction" (P/CC, 1997). To accomplish risk management decision-making, a decision among options for risk management controls requires that the decision alternatives for risk management be specified. The characterization of options often means that risk assessors are asked to calculate risks given one or more proposed risk management scenario. Risk managers use these scenarios, the inputs of benefit-cost assessments, and other information to make decisions about the best option for controlling or mitigating risks.

One of the principles of risk management is that the risk management analysis and the proposed risk control strategy should be commensurate with the level of risk. The reality of this principle in practice is that it also often relates to uncertainty about the magnitude of risk. Highly uncertain risk estimates sometimes lead risk managers to expend additional risk assessment resources in an effort to reduce the uncertainties before decisions about controlling the risks are taken.

From the risk manager's perspective, risk assessment is only one among several tools that can be used to inform the risk decision made by decision makers. Decision making by risk managers can call for benefits-risk assessments, risk-risk analysis, VOI analysis, or trade-off analysis as additional information useful to making decisions about managing risks. Decision analysis might be used to create a systematic and transparent decision making process that evaluates the importance of factors ranging from the objectively scientific to social values. For most scientific endeavors, "risk-informed" or "risk-based" decision making benefits from a formal decision analysis that provides a systematic analysis of complex scientific information, concerns of stakeholders, the constraints on risk management options caused by gaps in data, models or policies, and need for transparency in governmental decision making.

7.4 What are Some Other Inputs into Risk Management Decisions About Controlling or Accepting Risks?

Decision making about risks may require balancing results of a risk assessment with the results of benefit-risk and risk-risk tradeoff analyses, the need for risk management resources to address other risks in the agency's portfolio, and political pressures from stakeholders in industry, the public, or legislatures. The most important

input from risk assessment into the decision-making is a high-quality risk characterization from which risk managers can evaluate the scientific underpinnings of risk estimates, including a characterization of uncertainties in the estimates.

Risk managers make decisions under uncertainty. The results of benefit-risk and other analyses prior to risk management decision making often include equivalently uncertain estimates for the impact of proposed risk controls on the reduction or elimination of risks. Here, risk managers can apply formal decision analysis to trade-off decision alternatives, based on both objective and subjective assessments, for a transparent decision. In other situations, the decision to control or accept risks might be well-defined by existing regulation or guidance. Ultimately, the goal of risk management is to achieve an appropriate level of risk. The affirmation of this goal might not occur until after risk management controls are applied and the risks have been evaluated iteratively. Decision-analytic approaches, which are recommended by the NRC, evaluate the utility of specific policy options (NRC, 2009).

Historically one of the most difficult aspects of risk management for some types of regulatory functions is setting an acceptable or tolerable level of risk. Different approaches to setting standards have different ways of framing risk levels. The term "acceptable" is not generally used anymore, but has been used historically, so may be important for framing the context of the history within your agency. You should be aware of what approach has historical precedence in the field that applies to your risk assessment and your agency. A summary of some historical approaches to acceptability that have been suggested to regulators is provided below (adapted from Lowrance, 1976; Fischhoff et al., 1981; Lave and Romer, 1981; Humber and Almeder, 1986):

a) **Reasonableness** – This is a commonly cited principle in safety judgments. For example the Consumer Product Safety Commission is mandated to "reduce unreasonable risk of injury."

b) **Custom of usage** – The U.S. Food and Drug Administration (FDA) "generally recognized as safe" (GRAS) determination is for food substances which do not have to be regulated as additives because among other reasons they have a history of usage. Table salt and sugar are examples.

c) **Prevailing professional practice or professional judgment** – Originally established for physician's clinical practice, the principle is also used for local building standards and toy design. The underlying assumption is that sanction by custom is safer than untested. In using professional judgment, professionals rely on personal experience, accepted professional practice, and their clients' desires to judge risks.

d) **Best available practice, highest practicable protection, and lowest practicable exposure, best available technology** – Air and water quality regulations are associated with these standards, but they still require judgment. Lowest practical is also known as "as low as reasonably practical." (Vatn, 2004).

e) **Risk benefit (degree of necessity or benefit)** – A rough balancing of risks and benefits is attempted.

f) **The Delaney Clause or No-risk –** Named after former New York Congressman James Delaney who added language to the Federal Food, Drug, and Cosmetic Act that states "no [food] additive shall be deemed to be safe if it is found…to induce cancer in man or animal." The clause has been criticized for omitting other health endpoints and for ignoring dose-response relationships. This approach attempts to lower risks to zero.

g) **No observable adverse effect level (NOAEL) –** Because no adverse effects are observed at a given level of chemical, that level is deemed acceptable. It is customary in chemical risk that when the NOAEL is based on animal data, a safety factor (usually between 10 and 1000; 100 is a common factor) is applied to arrive at the level for humans. (The decimal point in the NOAEL is moved to the left to make the appropriate level lower.)

h) **Cost-effectiveness –** This approach equates the cost of saving lives or preventing adverse effects across programs.

i) **Formal benefit-cost analysis (BCA; also referred to as cost-benefit analysis) –** BCA is a quantitative analysis framework that incorporates the explicit dollar value of a human life or human well-being. Performance of cost-benefit analysis only serves to inform the risk management decision; it cannot determine the decision (Williams and Thompson 2004).

j) **Risk-risk –** This approach balances various risks against each other.

k) **Quantitative risk assessment –** The risk is expressed as a mathematical statement of the chance of illness or death after exposure to a specific hazard, and it represents the cumulative probabilities of certain events happening and the uncertainty associated with those events. Alternative management assumptions can be tested to evaluate the effect on the estimated risk. [43]

For chemical risk assessments, the risk level usually is stated quantitatively. When evaluating microbial risk, regulators very often refer to quantified risk reduction, without actually stating the level of risk associated with those risk reductions or commenting on the acceptability of the level of risk. Some levels of risk that are customary for microbial hazards in different media include:

a) **Ambient recreational water –** The U.S. and the most stringent European Union and WHO standards are associated with about one to two percent (1-2%) increased risk of gastrointestinal illness due to exposure to ambient water during recreational activities (EPA, 2004b).

b) **Foods –** Hazard Analysis Critical Control Point (HACCP) plans are common and are not linked to acceptable health risk levels, but are designed to ensure contamination of food is prevented or otherwise mitigated. Food Safety Objectives and Performance Objectives are stated goals (often numeric in nature) for public health, processing, transportation, or retail safety (Rieu et al., 2007;

[43] Proposed control measures must really reduce risks, not transfer them somewhere else (de Koning 1987).

Crouch et al., 2009). Management options are often linked to quantified public health outcomes.

c) **Biosolids** – Microbial standards are currently based on operational standards, which should provide pathogen levels that are below detection limits. The standards are not linked to level of health risk.

d) **Air** – There are no standards or Threshold Limit Values for microbial pollutants in the United States, but numeric criteria for mold and bacteria levels have been set in other countries. Indoor levels are compared to outdoor levels.

e) **Vaccinations** – Historically accepted or rejected risk levels of different adverse health outcomes are compared to benefit:risk analysis for new vaccinations (FDA, 1999).

f) **Occupational** – The General Duty Clause provides employees with workplaces that are "free from recognized hazards that are causing or are likely to cause death or serious physical harm." Target risk levels for quantified health outcomes are not discussed (29 CFR 1910.1030).[44]

7.5 What are Some Operational Risk Management Tools and Approaches?

A major reason for the development of the public health field was for the prevention of microbial diseases in human population caused by unsanitary conditions (e.g., John Snow and the 1854 cholera outbreak) or the presence of vectors for disease transmission. The regulatory tools at the disposal of the public health risk manager span an entire range of options to prevent the occurrence of pathologic organisms (e.g., *Clostridium botulinum* in low-acid canning) to limiting the means of primary or secondary transmission to vaccination of the host (e.g., for *Bacillus anthracis)* to improve host resistance to disease. Sanitary engineering designs that provide clean drinking water and separate waste water channels are credited with preventing countless outbreaks of disease (e.g., cholera, typhoid fever) and saving countless lives in more modern times. Even the education and outreach programs to promote safe handling of raw foods during preparation are part of risk management strategies to reduce the incidence of foodborne illness.

Generally speaking, operational risk management controls for health hazards are classified as physical, administrative and management controls (Table 7.2). For the particular case of microbial hazards, a "biological" classification for the host could be added for the possibility of immunization against some of the microbial hazards.

[44]http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

Table 7.2 Class of Risk Management Controls for Operational-Level Microbial Risk

Class of Operational Risk Controls	Examples
Administrative	• Engineering designs: process controls • Work practices and standards • Sanitary practices by personnel • Training program design
Managerial	• Training execution • Supervision • Appropriate skills for the tasks that might lead to contamination or favorable growth
Physical	• Barriers and physical factors that eliminate or reduce the chance of contamination and growth of pathogens • Pasteurization with heat or ionizing radiation • Packaging • Disposable gloves
Biological (specific microbes)	• Vaccination • Food animal treatments with benign organisms

7.6 What is Risk Management for the Intentional Use of Regulated Microorganisms?

Microorganisms whose intentional uses fall under federal laws including, but not limited to, the Federal Insecticide, Fungicide, and Rodenticide Act, the Federal Food, Drug, and Cosmetic Act, the Plant Protection Act, the Endangered Species Act, and the Invasive Species Act, often have specific risk management options available to the regulators. These options can involve placing restrictions on their use or, if justified, denial of permission for their use. Restrictions on their use can vary according to the specific law and regulations involved. For example, for pesticides the restrictions can be used to limit exposure by specifying on the approved labels exactly when, where, and how much can be applied. Furthermore, protective clothing, including respiratory masks, may be required to be used. Restrictions can also be set on what levels are acceptable to appear on food crops, although due to the potential for growth subsequent to application, microorganisms with any potential for human toxicity and/or pathogenicity are generally not allowed to be used on food crops. Containment requirements can be placed on intentional uses of regulated microorganisms for field testing and for industrial uses. Genetic engineering can be used to reduce risk concerns for intentionally used and regulated microorganisms, e.g., by deleting toxin genes. In addition, marker genes can be used to better identify the specific approved uses. Restrictions on the storage and movement of the regulated microorganisms can also be used to mitigate risk.

7.7 Summary

The objective of risk management is to protect human health. Risk management runs concurrently with risk assessment. Any decision that is policy related is usually made by risk managers and not risk assessors. Much of the scope of what the risk assessment covers is based on policy decisions regarding the scenario that is being modeled. Risk managers should work iteratively with risk assessors during the planning and scoping activities to collaborate on defining clear, scientifically defensible "risk questions" before the analytical components of risk assessment are executed. Risk management should follow a structured approach that is transparent, consistent, and fully documented. Risk managers are responsible for seeing the big picture, which means effective communication with all identified stakeholder groups.

Risk managers use the results of risk assessments to inform decisions that may also have broad social, economic, ethical, and political aspects. Risk manager's decisions may require trade-offs. You should understand that the risk managers will need to make decisions even when there are information gaps. You will need to ensure that the risk managers understand the uncertainties well enough that those uncertainties and their implications can be presented in lay terminology. Because information availability and scenarios can change, risk management decisions are reviewed, and revised when necessary.

8. RISK COMMUNICATION

A risk assessment is only as good as your communication of its output. All of the time and effort you spend conducting a risk assessment is for naught unless there is clear communication of risk assessment results to risk managers and other stakeholders. Effective risk communication requires diligence and has the same level of importance as any component of the assessment.

This guideline does not provide a detailed treatment or guidance for risk communication; you can refer to other places for such detail (Hallman, 2008; Sellnow, 2008; Morgan et al., 2002; Lundgren and McMakin, 1998). Rather, this chapter provides an initial understanding of how risk communication plays an important role in the risk assessment process.

Risk assessors are not expected to carry the load of risk communication. You need to work with the appropriate communication offices in your agency (e.g., public affairs office, Congressional outreach office). These communicators need to be part of the team during planning and scoping and throughout the remainder of the risk assessment process.

8.1 What is Risk Communication?

Risk communication is an iterative process that describes and exchanges information about risk, including its form and severity, and what can be done to lessen or avoid risk. It includes two equally important objectives:

a) Inform risk managers about risk so that they may make informed decisions.

b) Inform the public about risk so that they understand the nature of the risk and what is being done or will be done about it. (In most government agencies, communication with the public is done through a designated office.)

Risk communication is also the interactive exchange of information and opinions concerning risks and risk management among risk assessors, risk managers, consumers, and other interested parties (WHO, 2000). At its best risk communication results in informative and productive exchanges and can include joint problem-solving by legitimate stakeholders and the government.

8.2 What are the Aspects of Risk Communication?

Risk communication principles include (OMB, 2007b):

a) Risk communication should involve the open, two-way exchange of information between professionals, including both policy makers and “experts” in relevant disciplines, and the public.

b) Risk management goals should be stated clearly, and risk assessments and risk management decisions should be communicated accurately and objectively in a meaningful manner.

To maximize public understanding and participation in risk-related decisions, agencies should:

1) explain the basis for significant assumptions, data, models, and inferences used or relied upon in the assessment or decision;
2) describe the sources, extent and magnitude of significant uncertainties associated with the assessment or decision;
3) make appropriate risk comparisons, taking into account, for example, public attitudes with respect to voluntary versus involuntary risk; and,
4) provide timely, public access to relevant supporting documents and a reasonable opportunity for public comment.

The aspects of a good risk communication plan (see section 2.4.8) include:

a) Involvement and input of risk managers and stakeholders throughout the risk assessment process;
b) Clear risk management questions that are understood by managers and other assessors;
c) Awareness by managers and other stakeholders of the strengths and limitations of the assessment.

Social and personal behaviors are strongly influenced by risk managers' pronouncements, but only if the underlying risk assessment process is transparent to the public and considered credible. Trust is based on open communication and the credibility of provided information; thus, the public must be aware of the science behind the risk assessment. People who write risk communication statements should consider acknowledging both the power and the limits of the risk assessment process and the data used in the risk assessment.

8.3 Who are the Stakeholders of MRAs?

Stakeholders are people or organizations that may be affected by the relevant decision and thus, have an interest in the outcome of the risk assessment. They typically include people such as those in industry who will be responsible for implementing and will be financially affected by new rules and regulations borne of the risk assessment, as well as the general public. For example, a risk assessment that results in new regulations for *Escherichia coli* in ground beef would affect all persons who produce, consume, and handle ground beef. Anyone interested in a risk assessment may be reasonably termed a stakeholder. Stakeholder groups will self-identify but should also be sought out. Identification and communication with stakeholders can start with the planning and scoping process. Different stakeholder groups may have specifically tailored

communication materials. For example, susceptible populations may need more detailed information on what they can do to provide extra protection from the hazard.

Risk managers, assessors, and communicators are not typically considered stakeholders because they should remain unbiased. If the results of the risk assessment could affect them and the outcome was altered because of this knowledge, this would be considered a conflict of interest.

8.4 With Whom Can I Communicate?

Even if risk communication specialists are on the team, you as the risk assessor may serve in a risk communicator role and be responsible for communicating with stakeholders and developing outreach materials. If this is the case, you can work closely with appropriate agency offices to follow protocol. For example, many agencies will request that communication with members of Congress be done through a specific office. Communication strategies and materials for different stakeholders should be tailored to that particular audience. For example, communication with technical experts would be at a high level of technical detail, while communication with the lay public would be less technical. Communication materials targeted to many different specific audiences may be developed. Possible audiences include:

a) Technical experts – including microbiologists, mathematical modelers, experimental scientists, water treatment or food processing experts, or epidemiologists.

b) Lay public – including rate payers, community activists, activists focused on a particular disease caused or associated with the microbial risk (such as advocates for children, or persons with HIV/AIDS), environmentalists, or animal rights advocates.

c) Persons with financial or professional interests in either the status quo, or with the adoption of new technologies or techniques, which may be eventually preferred or mandated because of the risk assessment.

d) Governmental officials at the local, state, and national level and administrators who will have to enforce or monitor actions taken as a result of the assessment.

The language used in communication materials should conform to your agency's standards, but needs to be absolutely comprehensible by almost all being addressed. Agency communications, public affairs, and/or outreach offices should be consulted to assure that the message can be understood. Your agency may also have specific guidance for coordinating with other federal, state, and local health and environmental agencies.

8.5 When Can the Process of Risk Communication Begin?

There is a tendency to view risk communication as a final stage to the risk analysis process—something that occurs upon *completion* of the risk assessment and risk management. It is critically important that risk communication strategies be developed at the *beginning* of a risk assessment (during planning and scoping), to inform risk managers and other stakeholders *throughout* the risk assessment. The best place to start the risk communication process is during planning and scoping discussions in which a risk communication specialist can draft a risk communication plan (i.e., the communication strategy, including the risk assessor's role).

During development of the risk assessment, the team leader can be proactive in implementing the risk communication plan, particularly communicating with risk managers and stakeholders. One way to do this is by announcing that the agency plans to conduct a risk assessment. An announcement can be placed in the Federal Register. Other good venues for announcing risk assessments (and subsequent activities) include the agency's web site, advertising/announcement sections of trade and professional journals, and at professional meetings.

8.6 Can I Communicate in Writing, Orally, or Both?

Both. Oral communication is needed at many points throughout development of the risk assessment. It is appropriate to keep risk managers and other stakeholders informed about progress of the risk assessment. For example, you may wish to schedule a weekly (or other appropriately frequent) phone call with managers to keep them updated. Similarly, periodic conference calls or public meetings with stakeholders lessen the chance that they are caught off guard when the risk assessment is completed.

Communication in writing is almost always appropriate, especially since a written record is usually needed. For example, it is good to have the risk assessment questions in writing. Doing so entails back-and-forth work with risk managers to identify and clearly articulate the purposes of the risk assessment. By solidifying the questions in writing, you help ensure that risk managers, risk assessors, and stakeholders clearly understand what the risk assessment is intended to accomplish.

The most current risk communication message should be available in some written form (e.g., fact sheets), as well as electronically, throughout the process. Information should be available on your agency's website (although it should also be accessible to all who do not have computer access). Coordination with local health authorities in most cases is also important, and they may be able to suggest useful approaches to communicating with their public.

Virtually all completed risk assessments include a written report. The transparency, clarity, consistency, and reasonableness (TCCR) principles discussed and integrated throughout this guideline will help with the effective written communication in

the form of the report. In addition, many risk assessments are presented publicly as presentations or seminars, and the results can be communicated through social media.

8.7 Who Decides What to Communicate?

The risk manager—in consultation with the risk assessor—is responsible for deciding what information to communicate. Any formal communication or information release should be firmly based on the documented findings of the risk assessment. Therefore, you are responsible for communicating risk to the risk manager for a full understanding of the potential risks that would then be considered in the decision making process. Again, risk communicators can assist in the delivery and presentation of the information to the public.

MRA is an inexact science, requiring judgment calls and policy decisions on the part of highly trained, experienced professionals. This message should be included in any communication of risk, following a statement of known facts and preceding prescriptive risk reduction measures. Mathematical constructs and underlying assumptions should be made clear. Although there may be differences of opinion among risk assessors, the risk manager is ultimately responsible for deciding on a transparent and clear message on which the audience can evaluate agency actions.

8.8 What Information Can be Communicated?

The content of the formal risk assessment (as communicated to the public or to stakeholders) is determined by the risk manager, but you are responsible for presenting all available data, including those that challenge or do not support points in the assessment. The information that the risk manager may decide to communicate includes:

a) Data on human disease identified as either historical or experimentally-derived, or projections generated by modeling;

b) Underlying uncertainties or data gaps;

c) The degree of potential hazard to sensitive populations such as children, the elderly, and immune compromised people (focusing on susceptibility and severity);

d) Explanation that the different pathways that were deemed relevant were explored thoroughly;

e) The possibility of person-to-person transmission (if applicable);

f) Any results from animal testing and how these data may be relevant to humans;

g) The potential for zoonotic transmission between humans and animals (if applicable);

h) Potential actions to reduce exposures (for example, following posted signs regarding swimming, fishing, or harvesting clams).

You will have to provide the risk manager with your best professional judgment about the degree of risk associated with a specific hazard. This judgment can be accompanied by all relevant data about both the hazard and the target population. Probably some of the most important pieces of information you need to communicate are descriptions of the probabilities, uncertainties, and possible sources of biases or error (assumptions, generalizations) in any interim or final assessment. Communication of this information is important for the transparency of the risk assessment.

8.9 How is the Communication Process a Continuous Dialog?

Effective risk communication is a continuous process that requires constant feedback throughout the risk assessment. This begins at the planning and scoping stage, when all parties involved (including stakeholders as appropriate) should have a chance to comment on the need for a risk assessment, the scope of the risk assessment, and the other factors discussed in planning and scoping. Communicating effectively during planning and scoping helps to promote buy-in and helps to ensure respect for the risk assessment process. Communication of risk from assessors to managers is constant throughout the process. Communication of risk from risk managers to the public is usually episodic but, nonetheless, scheduled regularly.

As an example, interactive communication occurs between risk managers and risk assessors in developing risk management questions. A risk manager may say s/he wants a risk assessment to address "*Salmonella*." It is then up to you (as the risk assessor) to press for more specifics. At this point, perhaps the manager refines the question to "What is the effect of increased cooking temperatures on illnesses from Salmonella?" Then you may indicate that data are only available for a specific serotype, Enteritidis for example, but not all serotypes. Thus, the manager can work to refine the question to include Enteritidis only. In the end, iterative communication helps ensure clear and concise questions, which in turn increases the likelihood of a useful risk assessment. This iterative dialog can be conducted in person to speed up the process, but written documentation of the understandings between risk managers and risk assessors is important for clarity and the orientation of new team members.

Communication can also be iterative when describing results of the assessment to managers and other stakeholders. In virtually all cases, there is considerable room for improvement in risk assessments. Therefore, it is unwise to present results from risk assessments as if they were definitive. Instead, the results should be presented as a best effort, with the idea that feedback from managers and stakeholders will likely improve the assessment.

8.10 How In-Depth Can I Communicate?

Depth of communication depends on the audience. For example, if you are describing the risk assessment to another risk assessor, perhaps one who will peer review the assessment, then your communication should be very detailed. If, on the other hand, you are presenting the results to a high-level risk manager in the space of ten minutes, in-

depth details should be avoided unless specifically sought. When communicating with stakeholders, it is important to present results in a clear manner without talking down to the audience. For example, do not say, "This next part is complicated, so I'll put it in terms you can understand." Instead, start with a broad description of the work and then proceed to the details as both time and the audience's needs dictate. Lastly, it is very important to write a detailed report of the assessment. That way, even though you may be unable to give specifics during a talk, briefing, or other venue, you will always be able to refer your audience to the written report. The detailed report should be sufficiently detailed that another team of risk assessors with appropriate expertise can replicate the risk assessment. In addition to a formal detailed report, summary graphics, executive summary, fact sheets, and other types of overview documents can aid in communication efforts.

8.11 What Can I Do if the Message Is Not "Getting Through?"

It is incumbent upon you as a risk assessor to convey the results of the assessment. If you cannot do this, then the utility of the assessment is lessened. Accordingly, when you communicate the results of the assessment (or any aspect of the assessment for that matter), work to engage your audience. Take time to explore if your audience understands the points you are trying to communicate. If not, take a step back and work to clarify the parts of your message that are confusing. Look at this as an opportunity to exchange information and thoughts with your audience. Keep in mind that the difficulty may lie in your communication, not in your audience's comprehension. It may help if you communicate through various means. For example, in addition to speaking, it may help to take out pencil and pad and sketch your message. Be sure to allow time throughout your communication (be it a formal slide show or an informal conversation) for the audience to seek clarification. Regardless of whether you are experiencing problems getting through to the audience or not, consult communication specialists within your agency who can assist with communication in any situation.

8.12 How Can I Communicate Risk Successfully?

Successful risk communication requires strategic planning, skills, and practice. This planning requires in turn a very thorough review of the costs and benefits of specific actions (or inaction), and considers possible outcomes. Successful risk communicators consider the public to be stakeholders, inasmuch as agency decisions and actions affect them directly. The stakeholders base their own behaviors on information (however anecdotal) provided by trusted sources. Strategic risk communication practices may help to develop audience understanding and ultimately gain stakeholder and public cooperation. Again, consult with your agency communication specialists for assistance.

Strategic risk communication involves planning how to address stakeholder questions identified during planning and scoping and later during the assessment process. The responses to these questions should be straightforward and couched in simple language (rather than technical jargon). Concepts need to be packaged correctly, i.e., clearly, truthfully, and respectfully. This last requirement—that relevant concepts be

presented in understandable ways that enable discussion amongst all stakeholders, rather than as abstruse knowledge suitable for expert analysis only—is critical in establishing successful communication.

Risk managers turn to strategic risk communication when they need "buy-in." The best way to achieve cooperation of public and stakeholders in, rather than agitation against, risk-management decisions is by helping the audiences understand the options that involve cost (risks) and safety (benefits). Usually no one on the team can foresee the full range of audience responses. The starting relationship between agency and audience may be skeptical and/or confrontational, pitting audience experience against expert analysis. Strategic risk communication helps the audience to gain insight into the problem (and/or proposed actions) and to establish exactly what aspects of the proposed action (timing? approach?) are within their control. Ideally, successful risk communication will form the basis for mutual trust; but at the very least, strategic risk communication transfers information.

People respond not only to what is said, but how it is said. In addition, people respond to the way in which actions are carried out. Stakeholders value the qualities of listening, understanding, and responsiveness on the part of the agency. Other important factors include their own perception of risk acceptability, due process (in which stakeholders are able to participate in judging risks and predicted benefits), their personal sense of the risk-manager's credibility, and, above all, open communication (transparency). Successful risk communication is developed with full appreciation of the technical complexity of the situation in question, the controversy about or unavailability of the requisite science, the sensitivity of the communication environment, the potential relevance of political realities, and the perceived credibility of parties involved.

Other preparations can also contribute to successful communication. For example, practicing presentations before audiences such as co-workers can test the understandability of the presentation and increase the presenter's comfort level. The length of presentations is also an important consideration, since typically audiences do not want long presentations without opportunities to ask questions. Furthermore, practical information such as what actions can be taken by individuals or communities to interrupt exposures (e.g., beach closures to limit exposure to fecal contamination) can be included whenever the risk assessment is presented to the public, even if the actions suggested are not directly addressed or considered in the risk assessment.

8.13 How Can I Handle Media and Congressional Office Requests?

Risk assessors are sometimes approached directly with questions by external news sources and congressional offices. It is the responsibility of the risk manager and/or your communications offices (e.g., public affairs office, congressional liaison office), not the risk assessor, to communicate risk. If you are contacted directly from someone in the media or a congressional office, inform that person that your communications office is authorized to answer their questions (e.g., requests for interviews, background information, policy questions). Provide them with your communications office contact

information (phone number, e-mail address). If you are contacted directly via e-mail, simply forward the message to your supervisor and communications office. No matter how you are contacted, inform your supervisor and include as many details about the request as you can.

However, there will be occasions that, with appropriate permissions, you can provide technical information relevant to the risk assessment being queried. Remember that there is no such thing as "off the record." Assume everything said is on the record, even "background" information is still a response. Once you provide information, nothing really prevents the recipient from including that information in their article or report. A good reporter for example may not use "off the record" information as directly attributable to you, but will have a lead to contact others to verify it and then may use or release that information. You might also consider having a press officer on the phone or during interviews to make sure that the appropriate information is communicated. You may need to be aware of press deadlines and might be asked to contribute graphics to include in articles where appropriate.

8.14 When Can Risk Communication End?

Once a decision has been made, fully implemented, and openly communicated as per the risk communication plan, subsequent communication efforts may not be as intensive. However, there will likely be a need to monitor how the implemented decision is accomplishing its goal(s) or not, and communication will be critical at that time. You need to be aware that resultant actions based on the risk assessment may need to be re-evaluated and addressed further in the future (and this can be part of your communication plan). However, be careful not to mislead the public into expectations of involvement and re-evaluation that might not be appropriate.

Risk communication shouldn't have an absolute end; it can be an ongoing process, just as risk assessment is an iterative process. In another vein, risk managers can take the initiative to incorporate risk communication into routine functions. For example, the various microbial societies (most notably, in the United States, the American Society for Microbiology) produce informational outreach material targeting specific age groups and educational levels. These professional societies seek to expand public understanding of the role of microorganisms in human affairs beyond disease causation through staff dedicated to the effort. Agency public affairs offices might fulfill their responsibility to the public by teaming with these societies and by working with internal environmental-education staff to develop guidelines and procedures for ongoing risk communication. At least one senior public affairs manager could be tasked to work with agency experts to identify and communicate risk specific to children, seniors, and other sensitive populations. Interaction with public health and safety agencies (Centers for Disease Control [CDC] or the Occupational Safety and Health Administration [OSHA]) would be advisable, as would consultation with academic and industrial clinicians. A database of these and other external consultants can be developed in anticipation of potential outbreaks.

The risk communication network just described would be expected to develop outreach materials continuously, and to establish name recognition for the responsible agency via routine (e.g., bimonthly) public education broadcasts or activities. Such learning opportunities could be easily incorporated into local public school curricula, and ideally would be organized and distributed by agency offices nationwide. The network, once in place, can also provide informational materials to concerned individuals (and Internet blogs or other social media) or to news outlets.

8.15 Summary

Risk communication is ultimately the responsibility of the risk manager, who would work with internal public-affairs staff when available. It is your responsibility as the risk assessor to make the risk manager aware of the type and magnitude of the hazard involved, the population(s) likely to be exposed to the hazard, and the specific human- or ecological-health impacts resulting from this exposure. Direct interaction between you and the external affairs office (if responsible for public communications) would occur only with the risk manager's knowledge and approval, and ideally would focus only on technical issues. You should be aware early on who is responsible for monitoring the risk management response and communicating its effectiveness to internal sources.

Risk communication is an iterative process, if only because new information becomes available as the event of concern progresses. Mechanisms for information flow among the risk assessor, the risk manager, and any others involved in the communication effort should be developed as early as possible. Routine check-ins -- designed to make sure that all have the same, most current validated information -- must be implemented.

The need for risk communications specifically directed to different stakeholders, including susceptible populations, should be anticipated. Your responsibility would be to ensure that messages are clear, complete, and consistent, and to identify relevant external informational resources (such as state or local health authorities) where appropriate.

You should follow your agency's communication protocols, which usually means that external information requests are forwarded to the risk manager and/or the public communications team. You may be asked to report all external contacts both to your supervisor and to the risk management and communications team.

9. GLOSSARY

The term definitions in this glossary are from the EPA Thesaurus of Terms Used in MRA unless otherwise noted (EPA, 2007b). For the original sources of the definition, see the Thesaurus: http://www.epa.gov/waterscience/criteria/humanhealth/microbial/thesaurus/

acceptable risk
This is a risk management term. The acceptability of the risk depends on scientific data, social, economic, and political factors, and on the perceived benefits arising from exposure to an agent. Tolerable risk is a synonym.

analysis of variance
This is a statistical technique that isolates and assesses the contribution of categorical factors to variation in the mean of a continuous outcome variable. The data are divided into categories based on their values for each of the independent variables, and the differences between the mean outcome values of these categories are tested for statistical significance.

analysis plan
This is a plan that provides all the details of exactly how each part of the risk assessment will be performed. It usually describes in detail what analyses will be performed, how they will be performed, who will perform the work, schedules, resources, quality assurance/quality control requirements, and documentation requirements.

appropriate level of protection (ALOP)
Codex defines ALOP as the level of protection deemed appropriate by the member (country) establishing a sanitary or phytosanitary measure to protect human, animal, or plant life or health within its territory. The term is also used more broadly to refer to risk levels selected for regulations, rules, and risk assessments.

Codex
The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this Programme are protecting health of the consumers and ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and non-governmental organizations.[45]

conceptual model
Ecological risk assessment defines a conceptual model as a written description and/or a visual representation of actual or predicted relationships between humans or ecological entities and the chemicals or other stressors to which they may be exposed. ILSI (2000) states that a conceptual model depicts the purpose, defines the scope and scale,

[45] http://www.codexalimentarius.net/web/index_en.jsp

determines appropriate variables and identifies data needed for risk assessment. It can also serve as a preliminary or exploratory risk assessment.

cost-benefit analysis and cost-effectiveness analysis (CEA)
See OMB (2003) for full descriptions of cost-benefit analysis and CEA.

data objectivity
Data objectivity "focuses on whether the disseminated information is being presented in an accurate, clear, complete, and unbiased manner, and as a matter of substance, is accurate, reliable, and unbiased."(OMB, 2002)[46]

dose response
This is a relationship in which a change in amount, intensity, or duration of exposure to a pathogen is associated with a change in the manifestation and magnitude of human health effects.

dose-response assessment
This is the determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent, and the severity and/or frequency of associated adverse health effects (response).

dose-response curve
This is a graphical representation of the quantitative relationship between administered, applied, or internal dose of a chemical or agent, and a specific biological response to that chemical or agent.

dynamic model
This considers the individual within a community rather than the isolated individual. Time-dependent elements such as secondary transmission, host immunity, and animal reservoirs are included.

endpoint
For chemical risk assessment an endpoint is an observable or measurable biological event or chemical concentration (e.g., metabolite concentration in a target tissue) used as an index of an effect of a chemical exposure. For ecological assessment an endpoint is an explicit expression of the environmental value that is to be protected, operationally defined by an ecological entity and its attributes. For example, salmon are valued ecological entities; reproduction and age class structure are some of their important attributes. Together "salmon reproduction and age class structure" form an assessment endpoint. For MRA an endpoint is usually a health effect or infected state. However indicators or conditions associated with human health could also be endpoints.

[46] http://www.whitehouse.gov/omb/fedreg_reproducible/

epidemiology triad
This is the traditional model of infectious disease causation. It includes three components: an external agent, a susceptible host, and an environment that brings the host and agent together, so that disease occurs.

exposure
Exposure is contact made between a chemical, physical, or biological agent and the outer boundary of an organism. Exposure comprises the sources, mode, route, and extent of contact with the microbial hazard(s) of concern. How *often* a person is exposed is referred to as frequency of exposure. How *long* a person is exposed to a microbial hazard is referred to as the duration of exposure.

exposure assessment
This is the process of estimating or measuring the magnitude, frequency, and duration of exposure to a microbial hazard(s), along with the number and characteristics of the person or population exposed. The route of exposure is also considered.

exposure pathway
The exposure pathway is the physical and temporal movement of microorganisms from their *source* to the occurrence of an exposure. For chemicals the exposure pathway is the route a substance takes from its source (where it began) to its end point (where it ends), and how people can come into contact with (or get exposed to) it. An exposure pathway has five parts: a source of contamination (such as an abandoned business); an environmental media and transport mechanism (such as movement through groundwater); a point of exposure (such as a private well); a route of exposure (eating, drinking, breathing, or touching), and a receptor population (people potentially or actually exposed). When all five parts are present, the exposure pathway is characterized as “complete”, that is, capable of contributing to human health risks.

frank pathogen
A microorganism capable of producing disease in both healthy and compromised persons.

hazard
The term hazard can be interpreted in a number of ways. It may be defined as the stressor agent capable of causing an adverse effect on the recipient or the adverse effect itself. The selection of the definition is a policy decision driven by the existing statutes, regulations, or consistency with in-house processes. Codex considers a microbiological hazard is a hazard arising from bacteria, viruses, yeasts, molds and algae, parasitic protozoa and helminthes, and their toxins or metabolites.

Hazard Analysis Critical Control Plan (HACCP)
Seven basic principles are employed in the development of HACCP plans. These principles include hazard analysis, critical control point identification, establishing critical limits, monitoring procedures, corrective actions, verification procedures, and record-keeping and documentation. Under such systems, if a deviation occurs indicating

that control has been lost, the deviation is detected and appropriate steps are taken to reestablish control in a timely manner to assure that potentially hazardous products do not reach the consumer.

hazard characterization (HC)
In this guideline hazard characterization is the qualitative step of describing a microorganism's ability or potential to cause harmful effects. HC overlaps with HI, because both are qualitative descriptions of the hazard. In the Codex framework the term hazard characterization is a step in risk assessment that includes both qualitative description and quantitative description (e.g., dose response) of the hazard.

hazard identification (HI)
This is the process of determining if data support the case for a chemical or a microbe causing adverse health effects in humans and what those effects might be.

health effect
This is the clinical manifestation of disease associated with a specific pathogen, including symptomatic and asymptomatic infections, clinical illness, mortality, and sequelae.

health endpoint
This is an observable or measurable biological event used as an index to determine when a deviation in the normal function of the human body occurs.

host
This is a person or other living animal, including birds and arthropods, that affords subsistence or lodgment to an infectious agent under natural conditions. In an epidemiologic context, the host may be a population or a group.

host specificity
This is the characteristic of a pathogen that renders it capable of infecting one or more specific hosts.

immunocompromised
Immunocompromised individuals have a weakened immune system, making them more susceptible to infections than the general population.

incubation period
This is the time from the moment of inoculation (exposure) to the development of the clinical manifestations of a particular infectious disease.

indicator
An indicator is any biological entity or processes, or community whose characteristics show the presence of specific environmental conditions.

infectious dose
This is the number of organisms that make individuals ill or carriers. It should be noted that methods to count microbes may not be counting individual microorganisms. For example a colony forming unit (cfu) may be a clump of cells that formed one colony on a plate. An infectious dose is the minimum number of organisms that will result in entry through the host barriers, survival of the pathogen, and multiplication in the host. Infection may or may not result in symptomatic illness. On a population basis, there is no discernible minimum infectious dose for pathogens (FAO/WHO 2003). Instead there is a probability distribution for infection associated with different dose levels reflecting intra- and inter-individual variability in the pathogen-host relationship. Median infectious dose (ID50), which is the dose where half of a study group becomes infected, is a benchmark that is commonly used in animal studies.

infectivity
Infectivity describes the ability of a pathogen to enter, survive and multiply (infect) a host.

microorganism
These are viruses, bacteria, yeasts and simple fungi, single-celled algae, protozoa, all are defined as being organisms that can only be seen with the aid of a microscope. Most are beneficial but some produce disease (pathogens). Non-pathogenic microorganisms are critical for recycling energy and nutrients globally, such as in soil, the oceans, composting and sewage secondary treatment.

pathogen
These are microorganisms (e.g., viruses, bacteria, protozoa and the ova of helminth parasites) that can cause disease in humans, animals and plants.

pathogenicity
Pathogenicity refers to the ability of an organism to cause disease (i.e., harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions. Commensals and opportunistic pathogens lack this inherent ability to cause disease. However, disease is not an inevitable outcome of the host-pathogen interaction and, furthermore, pathogens can express a wide range of virulence. Virulence, a term often used interchangeably with pathogenicity, refers to the degree of pathology caused by the organism. The extent of the virulence is usually correlated with the ability of the pathogen to multiply within the host and may be affected by other factors (i.e., conditional). In summary, an organism (species or strain) is defined as being pathogenic (or not), and depending upon conditions, may exhibit different levels of virulence.

Pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease. Virulence is the disease producing power of an organism, the degree of pathogenicity within a group or species.[47]

[47] For further discussion of these terms visit http://scienceblogs.com/effectmeasure/2006/06/pathogenicity_virulence_transm.php

planning and scoping
This is the process that defines the purpose and scope of a risk assessment and focuses the issues and approach(es) involved in performing the assessment.

problem formulation
In ecological risk assessment, problem formulation is the initial stage of a risk assessment where the purpose of the assessment is articulated, assessment endpoints and a conceptual model are developed, and a plan for analyzing and characterizing risk is determined.
In microbial assessment, problem formulation is a systematic planning step that identifies the goals, breadth, and focus of the MRA, the regulatory and policy context of the assessment, and the major factors that will need to be addressed for the assessment.

qualitative risk assessment
Qualitative risk assessment uses verbal descriptors of risk and severity as well as uncertainty, and often involves the aggregation of expert opinions. The results are often stated in an estimated range, such as "there is a moderate to high risk of a certain outcome occurring."

quality-adjusted life year
This is a unit of health care outcomes that adjusts gains (or losses) in years of life subsequent to a health care intervention by the quality of life during those years. QALYs can provide a common unit for comparing cost-utility across different interventions and health problems. Other units for measuring health outcomes include DALYs and healthy-years equivalents (HYEs).

quantitative risk assessment
In quantitative assessments, the risk is expressed as a mathematical statement of the chance of illness or death after exposure to a specific hazard, and it represents the cumulative probabilities of certain events happening and the uncertainty associated with those events.

quorum sensing
Quorum sensing is a system of stimulus and response correlated to population density. Many species of bacteria use quorum sensing to coordinate gene expression according to the density of their local population.

risk analysis
A process consisting of three components: risk assessment, risk management and risk communication.

risk assessment
In the context of human health, risk assessment is a systematic way to prepare and organize information and help establish programs, R&D, and regulatory priorities; the qualitative or quantitative characterization of the potential health effects of particular

substances on individuals or populations; a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (4) risk characterization; the formal, scientifically based process to estimate the likelihood (probability) of exposure to a hazard and the resulting public health impact from this exposure. The product of the risk assessment is often a statement regarding the probability that populations or individuals so exposed will be harmed and to what degree (risk characterization).

risk characterization
In risk characterization, risk due to a particular exposure for a defined population is described in coherent, understandable, and informative conclusions about the microbiological risk to exposed humans in a way that is useful for decision makers as well as stakeholders in that risk. In all cases, major issues and uncertainty and variability associated with determining the nature and extent of the risk should be identified and discussed. The risk characterization should be prepared in a manner that is clear, transparent, reasonable, and consistent.

risk communication
This is the exchange of information about health or environmental risks among risk assessors and managers, the general public, news media, and other stakeholders. WHO considers risk communication to be the interactive exchange of information and opinions concerning risks and risk management among risk assessors, risk managers, consumers, and other interested parties (WHO, 2000).

risk management
In the context of human health, risk management is a decision making process that accounts for political, social, economic and engineering implications together with risk-related information in order to develop, analyze and compare management options and select the appropriate managerial response to a potential chronic health hazard.

risk profile
Developing a risk profile involves an initial systematic collection of information, which is evaluated to determine what other actions (including an MRA) and resources may be needed. The risk profile is an overall summary of the context in which a risk is being analyzed, including: a description of the risk(s) considered, values threatened by the risk, social perception of the risk, who benefits from producing the risk, who benefits from managing the risk, and characteristics of the risk, the risk-producer and the risk-bearer, which are pertinent to successful management of the risk.

secondary transmission
This is the direct or indirect propagation of a pathogen from an infected person (with or without clinical illness) to additional people.

sensitivity analysis
Sensitivity analysis examines the relative influence and importance of a model's inputs on its output measuring the 'relative' influence. It is the process of changing one variable

while leaving the others constant to determine its effect on the output. This procedure fixes each uncertain quantity at its credible lower and upper bounds (holding all others at their nominal values, such as medians) and computes the results of each combination of values. The results help to identify the variables that have the greatest effect on exposure estimates and help focus further information gathering efforts.

sequelae
These are abnormal conditions that arise following the acute phase of a disease. For example, kidney failure may follow acute *E. coli* O157:H7 infection.

stakeholder
A stakeholder is any organization, governmental entity, or individual that will be responsible for implementing, or financially affected by, new rules and regulations borne of the risk assessment, or may be impacted by a given decision based on the risk assessment.

superantigens
Superantigens are a class of antigens, which cause non-specific activation of T-cells.

susceptible, sensitive, vulnerable
These terms refer to individuals or populations that for varying reasons suffer more severe consequences than the general population as a result of exposure to a hazard. Although these terms are used interchangeably by many risk assessors and public health experts, the Interagency Risk Assessment Consortium Susceptible Populations Workshop considered the below definitions, but emphasized that when these terms are used, they should be defined.[48]
Susceptibility is: A capacity leading to higher risk at a given exposure level, due to biological (intrinsic) factors that can modify the effect of a specific exposure
Sensitivity is: A capacity for higher risk due to the combined effect of susceptibility (biological factors) and differences in exposure
Susceptibility - Includes intrinsic factors only; Characteristic of an individual; Defined by the host
Vulnerability - Includes intrinsic and extrinsic factors; Characteristic of an individual or a group; Defined by the host (behavior) and environment[49]

taxon
A taxonomic unit in the biological system of classification of organisms, for example: a phylum, order, family, genus, or species.

transparency
This is conducting a risk assessment in such a manner that all of the scientific analyses, uncertainties, assumptions, and science policies which underlie the decisions made

[48] http://foodrisk.org/IRAC/events/2010-01-10/downloads/Concept_of_Susceptibility-R_Parkin.pdf
[49] In this document vulnerability is also used in the context of a "vulnerability assessment," which is not related to the definitions discussed in this set of terms. Refer to Section 2.5.3 for the definition of vulnerability with respect to the CARVER method.

throughout the risk assessment are clearly stated (i.e., made readily apparent). For risk assessment to be transparent, methods, and assumptions should be clearly stated and understandable to the intended audience, so that the audience is able to evaluate the adequacy of the data and methods.

uncertainty analysis
This is used to estimate the uncertainty associated with model inputs, assumptions, and structure/form and the process of interpreting the influence of uncertainty on the results of a risk assessment.

uncertainty factor
These are usually applied to accommodate for a lack of knowledge associated with inter-species extrapolation, high to low dose extrapolation (i.e., effect to no-effect), population variation (i.e., protection of sensitive populations), and extrapolation across exposure durations (e.g., subchronic to chronic.) Although uncertainty factors are commonly applied in chemical risk assessment, much less information is available supporting the application of uncertainty factors to microbiological risk assessment.

uncertainty
Uncertainty is imperfect knowledge of the microbiological hazard (e.g., its virulence), environmental pathway/processes, or the human populations under consideration (from MRA). Uncertainty represents a lack of knowledge about factors affecting risk assessments and can lead to inaccurate or biased estimates of risk and hazard. Some of the types of uncertainty include scenario uncertainty, parameter uncertainty, and model uncertainty. Uncertainty can be reduced by further study.
NRC definition - Lack or incompleteness of information. Quantitative uncertainty analysis attempts to analyze and describe the degree to which a calculated value may differ from the true value; it sometimes uses probability distributions. Uncertainty depends on the quality, quantity, and relevance of data and on the reliability and relevance of models and assumptions.

variability
This refers to the observed differences attributable to true heterogeneity or diversity in a parameter. Examples include human physiological variation (e.g., natural variation in body weight, height, breathing rate, drinking water intake rate), weather variability, variation in soil types, and differences in contaminant concentrations in the environment. Variability is usually not reducible by further measurement of study, but it can be better characterized.
NRC definition - Variability refers to true differences in attributes due to heterogeneity or diversity. Variability is usually not reducible by further measurement or study, although it can be better characterized.

virulence
This 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. (see pathogenicity for comparison)

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Appendix A Example Assumptions

This appendix contains a list of types of assumptions that you may encounter during MRA. Many of these assumptions are due to data gaps. As data become available in the future, these assumptions could be different. Whether any of these assumptions are justifiable and adequate for a given risk assessment can be decided on a case-by-case basis. For example, the assumptions about the immune status of the population may be simple (no one has immunity) or complex (based on actual immunity data). Not all of these example assumptions will apply in all cases.

For all assumptions, the strengths, limitations, and implications of the assumption should be fully explored and documented in the risk assessment. This level of transparency helps peer reviewers decide if they agree with the basis of the assumptions and whether the results of the risk assessment seem credible.

Some assumptions are related to the scope of the risk assessment and are less subject to challenge on a scientific basis. For example the choice to evaluate a single agent instead of a mixture of agents is a policy decision about scope. In addition most MRA is limited in scope to estimating risk as a result of a single exposure event. In chemical risk assessment the exposure duration is often a 70-year life span.

1. General Assumptions:

MRAs focus on known pathogens that contribute significantly to the human disease burden or emerging pathogens for which the potential for disease is recognized, but the disease burden is unknown.

MRAs related to foodborne infections typically focus on a food-pathogen pair. This assumes that a specific pathogen is associated with a specific food. In reality, more than one pathogen could be associated with a specific food (for example, *Salmonella* and *Campylobacter* in chicken) and also different types of foods could be associated with a specific pathogen (for example, *Salmonella* in chicken and pork).

A given MRA's scope reflects the regulatory jurisdiction of the sponsoring agency and the statues behind regulations. For example EPA risk assessments in water media are divided into ambient water which is regulated by the Clean Water Act and drinking water which is regulated by the Safe Drinking Water Act. EPA has not to date sponsored an MRA that examined the risks of one pathogen in all water media. MRA for foods categories that are regulated by multiple agencies have required interagency collaborations.

A mathematical model is assumed to adequately represent complex biological phenomenon and ecological relationships.

Available data are assumed to be representative of the parameter. In practice, this assumption can lead to an overestimate or underestimate. Although MRA's routinely incorporate uncertainty (and/or variability) about the parameters of interest, the underlying assumption is that data included in a risk model is representative of the pathogen and target population of interest. Many examples illustrate the ubiquitous nature of this assumption. Data from experiments using animal models are commonly used rather than data from human infections. Certain related bacteria are considered to be surrogates for the pathogen of interest. Data based on short-term exposures are extrapolated to model the chronic effects of prolonged exposures. Public health surveillance data is considered to be reflective of the actual disease burden experienced by the human population under surveillance. Surveys of foods reflect the true prevalence and distribution of pathogens in that food. Surveys that focus on individual food consumption during a limited time period reflect the long-term consumption patterns of the individual, and the study population is representative of the population of interest.

It is assumed that it is appropriate to pool data derived from a variety of sources. This assumption can result in an overestimate or underestimate of true risk**.** Estimated prevalence and cell number distribution of pathogens is generally determined by combining results available in published literature, government's surveillance reports and industry reports. These data have inherent variability and uncertainty resulting from the variety of methods used to obtain them. It is assumed that pooling data from multiple sources (with or without weighting each observation with respect to a quality score) will provide a valid estimate of the parameter of interest with appropriate limits of uncertainty.

Normal distributions or triangle distributions are often assumed for parameters. Point estimates based on 50th, 75th, or 95th percentile are sometimes assumed and may not be correct or appropriate.

2. Assumptions concerning the Agent:

It is generally assumed that a minimum unit of the microbiological hazard is necessary to cause disease. For example, a risk model could be based on an underlying assumption that a single cell of a pathogenic bacterium has the potential to cause disease. Alternatively, it could be assumed that some minimum concentration of a bacterial toxin is required to cause disease.

Most models assume that each microbial unit acts independently, and that a single bacterial cell or viral particle has the potential to cause disease.

It is usually assumed that the chance of contracting a disease once an individual is infected is independent of the ingested dose, i.e., once infected, a particular individual contracts the disease regardless of ingested dose. A higher dose of pathogen would not cause more severe symptoms.

It is often assumed that factors intrinsic to the pathogen are the primary determinant of the agent's ability to cause infection/disease. Extrinsic factors (related to both the environment and the potential host) also impact the affect the pathogen's ability to cause infection/disease, and in some cases may be a more important determinant of the health outcome than the agent itself.

Certain assumptions must be stated regarding the variability among pathogen subtypes with respect to multiple characteristics that influence the development and/or detection of infection and/or disease. Many characteristics intrinsic to a specific pathogen ultimately influence both the probability that a specific subtype of a pathogen will result in an infection (or development of disease) and that this infection/disease will be detected and subsequently reported. These characteristics must be considered and prioritized according to their relative importance in impacting the health outcome of interest. In some cases, data is available that permits differentiation among subtypes with respect to these factors during the risk assessment process. For example, certain *Salmonella* serotypes appear to be more pathogenic to humans than others, and their relative pathogenicity could be defined to vary between serotypes in risk models. In other cases, these data are not available and it becomes necessary to assume that all subtypes are equal with respect to the defined characteristic. For example, certain *Salmonella* serotypes could be assigned the same relative pathogenicity in risk models when information that discriminates among the serotypes is unavailable. It is common to assume all isolates in the scenario are equally pathogenic.

Certain assumptions must be stated regarding the variability among pathogens and pathogen subtypes with respect to survival and growth in a variety of matrices. For food matrices in particular, many characteristics intrinsic to a specific pathogen ultimately influence the ability of the pathogen to survive and/or grow in that matrix. These characteristics must be considered and prioritized according to their relative importance in impacting the role of a given food as a vehicle. Assumptions concerning these characteristics are often necessary. For example, the pathogen may not be able to proliferate in the food, but it can survive and cause an adverse effect when consumed by a susceptible host. Some pathogens can form spores to ensure survival during adverse environmental condition and other may produce toxins under certain circumstances.

It is assumed that available growth kinetics models are adequately representative of all pathogen subtypes and are appropriate across different food matrices/environmental conditions. This assumption can lead to an overestimate or underestimate of risk depending on the actual growth kinetics profile of the pathogen within the situational food matrix or given environmental conditions.

3. Assumptions concerning the Host:

Certain assumptions are required with respect to the variability of susceptibility of individuals to the development of infection/disease. For example, it is frequently assumed that certain groups of people are more susceptible to infection/disease than others (i.e., young, elderly, pregnant woman, immunocompromised individuals).

Target population is assumed not to be vaccinated or immune due to previous exposures. The probability of infection or illness resulting from exposure is independent of previous exposures; also, the probability of infection or illness resulting from secondary transmission is also independent of previous exposures. This ignores the possibility of temporary or permanent immunity.

4. Assumptions concerning the Environment:

Risk assessment models typically assume that microbes are homogeneously distributed throughout the specified matrix. While this may be a reasonable assumption for certain foods (e.g., milk, juices) and air, and water, it is unlikely to hold true for most food categories or soil matrices.

MRA models typically assume that the quantitative levels of contamination (i.e. microbiological counts) are best represented by a log normal distribution.

Assumptions are stated concerning the analytical methods used to detect the presence/quantitative level of the pathogen in a matrix. It is typically assumed that viable pathogen can be detected (e.g., culture, bioassay, serological test, polymerase chain reaction [PCR]). It is necessary to indicate the limit of detection, analytical specificity, epidemiologic sensitivity, and epidemiologic specificity of a described method when interpreting the results obtained when testing to identify a particular pathogen in a defined matrix. It is often assumed that all subtypes of a pathogen are equally likely to be detected using a particular analytical method; however, this may not be valid. Similarly, certain methods are applied across a variety of matrices. In some situations, characteristics of the matrix itself may interfere with pathogen detection.

Assumptions concerning the geographic and temporal (seasonal) distribution of pathogens are required. For example, it is believed that *E. coli* O157:H7 exhibits a seasonal distribution in cattle, ground beef, and ambient waters. You could assume that this pathogen is more prevalent during May through September than in October through March when modeling potential exposures or you could assume that prevalence is uniform throughout the year. You could make similar assumptions concerning the geographic distribution of a pathogen.

The complex series of environmental events that impact the survival and/or growth of microbiological hazards are typically simplified in the context of risk models. Considerable variability exists with respect to environmental conditions (i.e., time, temperature, and pH) over time. For example, conditions associated with storage, refrigeration, product formulation, and batching process will vary greatly. Risk models assume that a single situation (or perhaps a limited number) occurs and that this factor occurs consistently over time.

5. Assumptions Concerning the Exposure Scenario:

The exposure time span of interest is usually specified in the scope. Whether the exposure time span is a specified event (e.g., meal, trip to the beach) or a lifetime it is still an assumption that must be transparent.

A specific food is the vehicle of transmission for a given pathogen. In reality, multiple foods may serve as a vehicle. Further, assumptions regarding the relative importance of potential exposures (i.e., foodborne; direct contact with animals, wildlife, insect vectors, or the environment; human-to-human transmission; water-borne exposures, recreational exposures) are necessary.

Certain assumptions are required with respect to individuals' exposures to pathogens of concern and the variability in exposures among individuals. These assumptions may lead to an underestimate or overestimate of risk. Consumer behaviors are diverse (e.g., by region, ethnicity, religion, food preparation, eating practices, packaging methods, manufacturing production practices, food production practices, local conditions, sanitation). Also, foods are not going to be consumed with the same frequency by the same people over an entire year. Factors such as seasonal availability of certain foods and changing eating habits may be appropriate to consider in a national scale MRA. It is not feasible or advisable to try to break out every possible behavior that may influence the exposure scenario. Often an average behavior is assumed to be representative at a population level (e.g., the number of servings consumed by each person or the number of contaminated servings).

Appendix B Hazard Identification Questions

This appendix contains examples of specific hazard identification questions that may be useful for the risk assessor's consideration. These are not all the questions risk assessors might consider. In addition, due to the nature of some of the questions, information gathering may need to be completed in collaboration with a public health or medical practitioner.

General Questions:

1. Which pathogens are of concern to public health? Which are regarded as being of *greatest* concern, and why?
2. Has the pathogen been identified?
3. What symptoms are manifest in the host and are they helpful in identifying the causative agent?
4. Prioritize and tabulate all the pathogens in terms of their degree of severity.
5. What is the hazard in question and what is the specific media or food of concern? (e.g., *Campylobacter* spp. in poultry).
6. What are the common routes of exposure associated with the hazard?
7. Are any media closely associated with, or often linked to, specific illnesses?
8. How is the media linked to the illness associated with the pathogen? (Is there epidemiologic evidence? What laboratory evidence exists?)
9. Are there available epidemiological data and microbiological data to associate what type of pathogen is associated with the medium of interest?
10. Are there adequate public health data to substantiate the occurrence of pathogenic microorganisms in the media in question?
11. Are there any established standards/guidelines regarding the pathogen of interest?
12. Are there data relevant to support the hazard? What information is there in peer-reviewed scientific literature? Are there existing databases from industry, government agencies, international organizations? What information is available from epidemiological, surveillance, or outbreak studies that can inform the assessment? Are there credible laboratory animal studies that have investigated the characteristics of the foodborne pathogen? Do relevant microbial ecological studies exist? Is there evidence of sensitive human populations (high risk, elderly, prenatal)?
13. For food media, is there a list of generic processes from the harvesting or slaughter to the table that represent "normal" exposures of the food product being examined through the food chain?

Questions concerning the Agent:

1. What type of pathogen is this organism (bacteria, virus, parasite, fungus, prion, noninfectious toxin)?
2. What are other taxonomic/strain considerations that influence ability to cause disease? Does the pathogen have particular strains that differ in ability to cause

disease? If so, what is the strain of interest? What is the subtyping of the pathogen?

3. What properties influence this agent's ability to cause disease?
4. What is the "life cycle" of this agent?
5. Does the pathogen produce a toxin? If so, is it the toxin that presents the hazard, or the pathogen? Is the pathogen an anaerobic, gram-positive, spore- forming rod that produces a toxin?
6. Are there indicator organisms or surrogate species that can allow for an indirect evaluation of this agent in the absence of data?
7. How is the pathogen identified? What are the biochemical/taxonomic characteristics to identify the pathogen?
8. What methods are available for detecting and quantifying the agent?
9. What are the detection methods to identify the foodborne pathogen?
10. What are the sampling and enrichment techniques to identify the foodborne pathogen?
11. Is there a microbiological testing/identification method for the pathogen in human clinical samples? Is there a specific method for testing for this pathogen?
12. What factors influence the spatial distribution of this agent (clumping, aggregation, particles, clustering)?
13. Is the pathogen a microaerophilic organism?
14. Are there phenotypic characteristics that influence virulence and/or pathogenicity?
15. Are there genotypic characteristics that influence virulence and/or pathogenicity?
16. What are the particular physical or chemical factors that the organism is sensitive to such as temperature, pH, oxygen availability, disinfectants, desiccation, ultraviolet or ionizing radiation, heat, or food preservatives?
17. Are the spores heat resistant? Can the spores survive in treatment processes?
18. What are the growth requirements of the organism? Is the organism free-living or is it an obligate parasite? What are the physical and chemical requirements for growth?
19. Can the organism replicate in the medium of concern?
20. What is the main pathogenic strain of this organism, and what other specie(s) within the identified genus are humans susceptible to?
21. What are the genotypic factors and phenotypic expression that influence this agent's virulence and/or pathogenicity?
22. What is the disease manifestation caused by this organism in humans? Under what conditions is the disease commonly expressed in humans, and what are the long term effects of the disease (sequelae) of the disease? Are there useful animal models to help address this issue?
23. How does the agent cause pathology and/or disease?
24. Does the microorganism produce a toxin while growing in the intestinal tract or other tissue?
25. What is the incubation period following exposure until the onset of the disease?
26. What is the mechanism of action for infection and illness?
27. Is secondary transmission possible? How contagiousness is the disease?
28. What is the target organ(s)?

Questions concerning the Host:[50]

1. Is there a population, life stage or other distinguishable group that is most susceptible to the disease (i.e., more susceptible than the general population)?
2. How wide is the host range of the pathogen?
3. Are any practices/behaviors closely associated with, or often linked to, specific illnesses?
4. Is illness host-specific, i.e., more likely to affect susceptible populations?
5. Are there general socioeconomic strata associated with the presence of the organism?
6. What is the nature of identified cases of infection? (e.g., sporadic, small/family related outbreaks)?
7. Are there behavioral and consumption practices associated with this pathogen and media?
8. Does the pathogen produce all or any of the following symptoms: abdominal cramps, nausea, vomiting, diarrhea, fever, dehydration? Or, does the microbe usually have no manifestations?
9. Is the pathogen found in the large intestinal tract only?
10. Does it shed in the feces?
11. What is the associated morbidity/mortality?
12. How might one define immunocompromised?
    a) Are there specific quantifiable biomarkers of immunocompetency?
    b) Can one define such biomarkers?
13. How would one account for genetic/ethnic/cultural differences within or between populations?
14. What is the definition of biomarkers for malnutrition?
15. Should age be defined with respect to chronology or physiology?
16. What would one do if the definitions [for different biomarkers] overlap?
17. What are the definitions for chronic ailments?
18. What are multiple concurrent factors?

Questions concerning the Environment:

1. What are the environmental conditions of the pathogen in question such as contamination of water or food, growth characteristics, inactivation, elimination, survival, for each part of the exposure scenario?
2. Does the pathogen have any characteristics that may promote its survival through the exposure scenario?
3. How do various environmental conditions (temperature, nutrients, pH) influence this agent's growth, survival, and/or death?

[50] Some questions concerning the host may be confidential medical information and the resulting answers may need to be protected. This would be particularly important if the affected population under investigation was small in size and the retrieved information could be linked back to the affected individuals.

4. Are there certain environmental conditions and/or control processes that this agent can survive (or develop resistance)?
5. Are there seasonal, geographic or climatic factors that affect the occurrence of this agent? What is the geographic location? What season does it occur?
6. What are the time factors involved in identifying the pathogen?
7. What are the temperature factors involved in identifying the pathogen?
8. If carried by another species, what is the geographic range of that species?
9. Is there a reservoir for the pathogen? Identify and list all the potential sources (reservoir) for all of the pathogens of interest.
10. What are the ways in which the pathogen can contaminate the media?
11. Is that pathogen zoonotic? Is the pathogen found in humans or animals or both?
12. Where (in what medium?) is the pathogen commonly found? Does the pathogen grow in the medium?
13. Is the pathogen endemic to a specific region or environment?
14. Has it been identified in other countries recently?
15. What are the reservoirs and/or environmental niches for this agent? Are there other important ecologic factors that influence this agent?
16. How can the media become contaminated with this pathogen? Are there any specific practices/behaviors that could promote survival of this pathogen?
17. For food, did contamination occur during growing, harvesting, processing, storing, shipping, or final preparation?
18. What is the route of contamination and location of the pathogen in the medium?
19. What is the microbial ecology to identify the pathogen?
20. What method(s) was used to identify the pathogen?
21. For food, what is the effect of food processing and preparation on their survival?
22. What are the marketing and preparation practices associated with this foodborne pathogen?
23. What are the globalization trends associated with this pathogen regarding the medium of interest?
24. Is the pathogen's presence in contaminated media the result of an error or breakdown in normal controls?
25. For food, identify and document all the associated stages and locations/residing of critical control points, and the time that certain pathogens may have been present.
26. What is the principal reservoir of this hazard, and where is it also commonly found?
27. What are common environmental media and the expected concentration of this agent?
28. Can the pathogen be found in animal feces?
29. Is the foodborne pathogen found in soil, dust, sewage, or intestinal tracts of animals and/or humans?

Questions concerning Transmission:

1. What are the possible modes of transmission? Could transmission occur through water, contaminated carcasses, fomites or other media?

2. Identify all the potential routes/pattern of transmission (direct or indirect) of microbial infection (disease) for each pathogen
3. Is the pathogen found in putting something in the mouth that has been contaminated with the stool of an infected person or animal; direct contact with the droppings of infected animals?
4. Is the pathogen a person-to-person transmission such as in child daycare settings?
5. For food media, what are the eating habits associated with the pathogen?
6. How does this agent infect a susceptible host? What are the typical routes of infection and/or portals of entry?
7. Is there potential for secondary spread?
8. What is the infectious dose?
9. Is there a dose-response model available for this organism?